TRUE ABSORPTION OF SELENIUM IN DAIRY COWS: STABLE ISOTOPE TRACER METHODOLOGY AND EFFECT OF DIETARY COPPER.

By

KAREN MARIE KOENIG

B.Sc., The University of British Columbia, 1984

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Department of Animal Science

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1988

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Animal Science

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date March 31, 1988

ABSTRACT

Gas chromatography mass spectrometry (GCMS) and inductively coupled plasma mass spectrometry (ICPMS) were evaluated for the measurement of selenium (Se) and Se stable isotope ratios. GCMS and ICPMS were found to be accurate for quantitative Se analysis in biological matrices by isotope dilution using Se-78 and Se-76 as internal standards, respectively. A higher precision was obtained for ICPMS than GCMS enabling a smaller quantity of the tracer to be administered to subjects in labelling experiments. The isotopes of choice for metabolic tracers were Se-76 when sample analysis was by GCMS and Se-77 and Se-82 when analysis was by ICPMS.

The influence of copper (Cu) on endogenous fecal Se excretion and true absorption of Se in nonlactating Holstein cows was examined by the use of Se stable isotopes as tracers. The method involved the application of conventional balance techniques in conjunction with isotopic enrichment of the body Se pools. Selenium in several tissues following oral and intravenous routes of isotope administration were evaluated as the precursors of endogenous fecal Se.

Two cows fed a Se deficient diet (0.035 mg kg^{-1}) were administered 4 mg Se-76 orally, daily, for 5 d. After a 10-d equilibration period total collection of feces was made daily for two 5-d periods. The animals were then sacrificed and samples obtained from all major tissues and fluids. Se-76 enrichment

(tracer/tracee mass percent, TTMP) in tissues was variable (< 0.56 - 13.4). However, enrichment was similar (9.8 - 12.9) in the tissues considered as potential contributors to endogenous fecal Se (serum, epithelium of the stomach, liver, bile, pancreas, small intestine and colon). Enrichment in serum and liver was used to calculate endogenous fecal Se. Apparent absorption of Se in the two cows was negative (-37 and -147 μg d^{-1}). Correction of apparent absorption for the fecal Se of endogenous origin gave a true Se absorption (% of intake) of 10 and 16%. The percentage of total fecal Se of endogenous origin was 23 and 36%.

In two trials, 5 or 6 cows were assigned to one of two Cu-supplemented treatment diets: 0 mg kg^{-1} or 17 mg kg^{-1}. The basal diet contained 0.19 mg Se kg^{-1} and 13 mg Cu kg^{-1}. To each cow ~4.6 mg Se-77 and ~1.3 mg Se-82 were administered by oral and intravenous routes, respectively. After a 14-d equilibration period, total collection of feces and urine were made daily for two 5-d periods. Serum was collected on the first, third and fifth days of each period. Liver biopsies were taken 2 d following the completion of the balance periods. The estimates of endogenous fecal Se (μg d^{-1}) from enrichment in the serum (256) and liver (235) following oral administration of the tracer and from enrichment in serum (241) following intravenous administration were not significantly different ($P>0.05$) but were higher than the estimate from the enrichment in liver (197) ($P<0.05$). No significant differences ($P>0.05$) were present when

true absorption ($\mu g\ d^{-1}$) was determined from enrichment in serum (290) or liver (268) following oral administration or from enrichment in serum (274) or liver (230) following intravenous administration. It was concluded the analysis of serum or liver with oral administration or the analysis of serum with intravenous administration of the tracer would provide reliable methods for estimation of endogenous fecal Se and true absorption. There was no effect of Cu on endogenous fecal Se excretion or true absorption of Se. Apparent and true absorption were 3.2 and 11%, respectively. Approximately 90% of the total Se excreted was in the feces, of which, 9.7% was of endogenous origin. The use of Se stable isotopes as metabolic tracers in dairy cattle provided a safe alternative to the use of radioactive tracers and enabled experiments requiring multi-isotopic enrichment to be performed.

TABLE OF CONTENTS

Page

Abstract. ii

Table of Contents v

List of Tables. viii

List of Figures x

List of Appendix Tables xi

Acknowledgements. xii

1. INTRODUCTION. 1

2. LITERATURE REVIEW 7

2.1. Selenium metabolism in ruminants. 7

2.2. Stable isotopes as tracers for the study of mineral metabolism 45

3. MATERIALS AND METHODS 52

3.1. Analytical techniques 52

3.1.1. Gas chromatography mass spectrometry. 53

3.1.2. Inductively coupled plasma mass spectrometry. 56

3.2. Calculation of Se stable isotope enrichment . . 59

3.3. Trial I 63

3.3.1. Animals 63

3.3.2. Diet. 64

3.3.3. Single isotope enrichment of the whole body Se pool. 64

3.3.4. Balance periods 65

3.3.5. Tissue collection 67

Page

3.4. Trial II and III. 68

3.4.1. Animals 68

3.4.2. Experimental diets. 68

3.4.3. Double isotope enrichment of the whole body Se pool. 70

3.4.4. Balance periods 73

3.4.5. Liver biopsy. 73

3.5. Calculations for apparent absorption, balance, endogenous fecal Se and true absorption 74

3.6. Experimental design and statistical analysis. . 75

4. RESULTS 78

4.1. GCMS analytical technique 78

4.2. ICPMS analytical technique. 83

4.3. Trial I 90

4.3.1. Animals and diet. 90

4.3.2. Selenium content of tissues and fluids. 90

4.3.3. Distribution of Se-76 in tissues and fluids. 93

4.3.4. Absorption and endogenous fecal excretion of selenium 100

4.4. Trial II and III. 104

4.4.1. Experimental diets. 104

4.4.2. Feed and water intake and urine and feces excretion 104

4.4.3. Selenium concentration in serum and liver 108

4.4.4. Isotope enrichment in tissues and excrement 108

Page

4.4.5. Apparent absorption and balance of selenium. 111

4.4.6. Endogenous fecal excretion and true absorption of selenium. 113

DISCUSSION. 119

5.1. Selenium stable isotopes as tracers 119

5.2. Selenium content of the experimental diets. . . 124

5.3. Tissue distribution of selenium 125

5.4. Method for measurement of true absorption . . . 128

5.5. Apparent and true absorption of selenium. . . . 136

5.6. Selenium excretion. 139

5.7. Effect of copper on endogenous fecal excretion and true absorption of selenium 140

6. CONCLUSIONS 145

7. BIBLIOGRAPHY. 148

8. APPENDIX. 162

LIST OF TABLES

Table		Page
1	Selenium concentration of tissues and fluids in cattle of adequate selenium status	23
2	Selenium concentration in tissues indicating marginal and deficient selenium status in cattle.	24
3	Stable isotope composition of chemical elements of concern to mineral nutrition	47
4	Description of tissue and fluid samples collected (Trial I).	69
5	Mass of Se-77 and Se-82 administered to each cow in Trial II and III	72
6	Selenium determination of standard reference materials by GCMS ($\mu g\ g^{-1}$)	81
7	Precision of selenium stable isotope enrichment determined by GCMS	82
8	Analysis of standard reference materials for selenium by ICPMS	88
9	Precision of selenium stable isotope enrichment determined by ICPMS.	89
10	Composition of orchardgrass hay (dry matter basis) (Trial I).	91
11	Selenium concentration in tissues and fluids of cow 8219 and cow 7939.	95
12	Estimated selenium content in tissues, fluids and the whole body of non-lactating dairy cows	96
13	Selenium content in the placenta and tissues of the 5-month old fetus from cow 8219.	97
14	Feed intake and feces and urine excretion in Period 1 and 2 (Trial I).	101
15	Intake and feces excretion of selenium ($\mu g\ d^{-1}$) (Trial I).	102
16	Apparent and true absorption of selenium ($\mu g\ d^{-1}$) (Trial I).	103

Table Page

17 Composition of the basal diet used in Trial II and III (dry matter basis) 105

18 Copper concentration of the experimental diets (dry matter basis) (Trial II and III). 106

19 Body weight, feed and water intake and feces and urine excretion for cows on Treatment 1 and 2 (Trial II and III) 107

20 Selenium concentration ($\mu g\ mL^{-1}$) in serum 1 day prior to the trial and during Period 1 and 2 (Trial II and III) 109

21 Se-77 and Se-82 enrichment (TTMP) in serum, liver, feces and urine (Trial II and III) 110

22 Apparent absorption and balance of selenium ($\mu g\ d^{-1}$) (Trial II and III) 112

23 Endogenous fecal selenium ($\mu g\ d^{-1}$) (Trial II and III). 115

24 True absorption of selenium ($\mu g\ d^{-1}$) (Trial II and III) 116

25 Effect of route of isotope administration and tissue analyzed on the calculation of endogenous fecal selenium ($\mu g\ d^{-1}$) (Trial II and III) 117

26 Effect of route of isotope administration and tissue analyzed on the calculation of true absorption of selenium ($\mu g\ d^{-1}$) (Trial II and III) 118

LIST OF FIGURES

Figure		Page
1	Excretion of ^{75}Se during a 12-day period following oral and intravenous administration of the isotope by lambs with varying selenium intakes	19
2	Selenide metabolism.	33
3	Possible mechanism of glutathione peroxidase	40
4.	Calibration curve for selenium standards enriched with Se-76 plus 5 levels of Se-78 ranging from $TTMP_{78}$ 0 to 83.9	79
5	Calibration curve for selenium standards enriched with Se-78 plus 5 levels of Se-76 ranging from $TTMP_{76}$ 0 to 11.8	80
6	Effect of HCl and Cu on the intensity of the Se signal	84
7	Se-76 enrichment (TTMP) in tissues and fluids of cow 8219 and cow 7939.	98
8	Se-76 enrichment (TTMP) in the placenta and tissues of the 5-month old fetus from cow 8219	99

LIST OF APPENDIX TABLES

Table | Page

1 Isotopic composition of natural abundance selenium and of the enriched selenium stable isotope preparations (atom percentage) 162

ACKNOWLEDGEMENTS

I would especially like to thank to Dr. W. T. Buckley, Research Station, Agassiz, for his invaluable advice and guidance provided throughout the conduct of this research. Many thanks to Dr. J. A. Shelford, Department of Animal Science, U.B.C., for his input and support.

I also wish to express my gratitude to D.V. Godfrey and E.G. Wilson, Research Station, Agassiz for their assistance with the chemical analysis of samples and to S. Hainstock, Research Station, Agassiz, for her assistance with the animal experiments.

The research was supported in part by a grant from Agriculture Canada and from a G.R.E.A.T. award #69(GC-5) from the Science Council of British Columbia.

1. INTRODUCTION

The most critical time for meeting the selenium (Se) requirements of the dairy cow extends from late gestation through early postpartum. It is during this period when conditions due to Se deficiency can develop in both the dam and offspring (Jenkins et al. 1974; Harrison et al. 1984).

In the mature dairy cow retained placenta, the failure of the fetal placenta to separate from the maternal placenta, is an expression of Se deficiency (Trinder et al. 1969; Julien et al. 1976a, b). Prepartum Se supplementation by injection (Trinder et al. 1969; Julien et al. 1976b) or in the diet (Julien et al. 1976a) has been reported to reduce the incidence of placental retention. The efficacy of the Se treatment appears to be dependent on the Vitamin E (Vit E) status of the animal. A "sparing effect" or synergistic relationship between Se and Vit E acts in the prevention of many of the various Se responsive disorders (Jenkins and Hidiroglou 1972).

Selenium deficiency during the prepartum period has been reported to predispose the dairy cow to increased risk of metritis and cystic ovaries (Harrison et al. 1984). There is some indication that deficiencies of Vit E and possibly Se elevate the incidence of mastitis from enviromental pathogens (Smith et al. 1984). A dietary deficiency of Se was found to increase the duration of the clinical symptoms of mastitis (Smith et al. 1984).

In dairy cattle of all ages a Se responsive condition known as unthriftiness or ill thrift may develop. At the subclinical level there is a failure to achieve optimal growth rate and production. Progression of the condition can lead to clinical symptoms such as a rapid loss of weight and mortality (Underwood 1981).

An inadequate Se intake by the dam increases the susceptibility of the developing calf to nutritional muscular dystrophy (NMD) (Jenkins et al. 1974). NMD, also known as white muscle disease, is a cardiac and skeletal myopathy which usually affects the young bovine in the first 1 to 3 months of life. A congenital form may also be found in animals at birth and in fetuses that have been aborted (Jenkins and Hidiroglou 1972).

There is increasing evidence of the importance of the involvement of Se in the immune system. Boyne and Arthur (1979) have shown that polymorphonuclear neutrophils of Se deficient cattle were unable to kill ingested cells of the microorganism Candidia albicans. Circulating neutrophils play an important role in the defense of animals against microbial infections. The role of Se in this function may underlie the mechanism whereby Se adequate dairy cows better resist the onset of clinical uterine infections and mastitis (Harrison et al. 1984; Smith et al. 1984). In addition to impaired neutrophil function and resistance to microbial and viral infections, Se deficiency has also been shown to inhibit antibody production, proliferation of T and B lymphocytes in response to mitogens, and cytodestruction

of T lymphocytes and natural killer cells (Kiremidjian-Schumacher and Stotzky 1987).

The current recommended dietary intake of Se by dairy cattle is 0.1 mg kg^{-1} feed dry matter (DM) (NAS-NRC 1978). If feed containing < 0.1 mg Se kg^{-1} is fed to dairy cattle, Se/Vit E responsive disorders may develop in varying degrees with a greater incidence occurring when the Se concentration drops below 0.05 mg kg^{-1} (NAS-NRC 1983). In a survey of feedstuffs grown in British Columbia, Miltimore et al. (1975) reported that the percentage of samples with a Se concentration below 0.1 mg kg^{-1} were: wheat, 12%; barley and oats, 32%; legumes, 22%; grasses, 21%; and corn silage, 76%. Selenium analysis of dairy cattle feed in the Upper Fraser Valley revealed lower Se values for all classes of feed than those found by Miltimore et al. (1975) (Cathcart et al. 1980). Eighty percent of the commercially prepared concentrates and only 2% of all other feedstuffs including pasture, grass silage, orchardgrass hay and corn silage were above 0.1 mg Se kg^{-1} (Cathcart et al. 1980). The British Columbia Ministry of Agriculture and Fisheries reported 14% of dry roughages and silages grown in the Fraser Valley contain > 0.1 mg Se kg^{-1} (Soder 1984).

Interaction of Se with other dietary nutrients can also precipitate Se deficiency in animals. Copper (Cu), silver, tellurium, zinc, arsenic, cadmium, lead, mercury and under some circumstances sulfur can induce typical lesions of Se/Vit E deficiency in animals fed diets containing amounts of Se

ordinarily considered adequate (Van Vleet 1980; Puls 1981).

Many of the dairy cattle feedstuffs produced in British Columbia contain levels of Cu below the recommended dietary level (10 mg kg^{-1}, NAS-NRC 1978) thereby leaving cattle susceptible to Cu deficiency. In addition to low levels of Cu in the diet, higher than normal contents of sulfur, molybdenum, cadmium, iron and zinc can induce Cu deficiency (Puls 1981). In an effort to meet requirements and avoid problems with interacting nutrients, Cu supplementation may reach quantities many times greater than the recommended dietary level.

High dietary Cu levels have been shown to be effective in overcoming the effects of Se toxicity (Hill 1974; Jensen 1975a). The existence of an interaction between Cu and Se at high Cu and adequate Se intakes however, is not as clear. High levels of dietary Cu (800 - 1600 mg kg^{-1}) when added to diets adequate in Se produce Se deficiency lesions in chicks (Jensen 1975b) and ducklings (Van Vleet and Boon 1980). The Cu was thought to reduce the availability of Se in tissues for synthesis of the selenoenzyme glutathione peroxidase by interfering with Se absorption and/or by formation of insoluble intracellular Se compounds. In contrast to these studies, White et al. (1981) found no effect of lower levels of Cu (10 mg kg^{-1}) on the metabolism of radiolabelled selenomethionine in sheep. There was however, a tendency for Cu to decrease the total retention of radioactive Se.

In view of the importance of Se to the health and well-being

of the dairy cow, an understanding of Se nutrition and metabolism and the possible interacting effects of other dietary nutrients are paramount for ensuring Se requirements are met. Experimental investigations of Se nutrition and metabolism in ruminants have been carried out by measuring total elemental Se and/or using radioactive ^{75}Se. ^{75}Se has been used successfully as a metabolic tracer in ruminant animals (Handreck and Godwin 1970; Kincaid et al. 1977; Symonds et al. 1981a, b) but it does present limitations for use in metabolism studies with mature dairy cattle. The isotope has a relatively long half-life and the associated radiation hazards can make handling and disposing of excrement, fluids and carcasses a problem. The use of stable isotopes as tracers overcomes these disadvantages associated with using radioisotopes in large animals. In addition there are six stable isotopes of Se thus enabling multi-isotopic studies to be performed.

To aid in the understanding of Se metabolism and nutrition through the application of non-invasive, non-hazardous tracer methodology, this research was conducted to evaluate the use of Se stable isotopes as tracers in dairy cows. The measurement of Se and Se stable isotope ratios by gas chromatography mass spectrometry (GCMS) and inductively coupled plasma mass spectrometry (ICPMS) with sample introduction by hydride generation were evaluated. The effect of Cu on true absorption of Se in dairy cows was determined by utilizing a method combining Se fecal balance with measurements of endogenous fecal

excretion in cows with an isotopically enriched whole body pool of Se. Oral and intravenous routes of isotope administration and the analysis of several tissues were evaluated for the estimation of endogenous fecal excretion and true absorption of Se. The tissues analyzed were selected based on the tissue distribution of an enriched Se stable isotope.

2. LITERATURE REVIEW

2.1. Selenium metabolism in ruminants

Interest in the biological significance of Se began with the recognition of its toxic and possible carcinogenic properties. It was not until 1957 that work by two independent groups demonstrated the essential physiological role of Se. Schwarz and Foltz (1957) first demonstrated supplementation of Se to rats on certain diets would prevent the development of liver necrosis. In the same year Patterson et al. (1957) showed Se also prevented exudative diathesis in chicks. This sparked research and investigation into the nutrition and metabolism of Se in a number of animal species in many areas of the world. It led to the recognition of a number of Se/Vit E responsive diseases in all classes of livestock and poultry.

Disorders associated with Se and/or Vit E deficiency include: exudative diathesis, encephalomalacia, myopathy, and decreased egg productivity and hatchability in poultry; breast, heart, intestinal and gizzard (white gizzard disease) muscle dystrophy and poor reproductive performance in turkeys; myocardial degeneration and necrosis (mulberry heart disease), hepatic necrosis (hepatosis dietetica), nutritional muscular dystrophy, circulatory failure, gastric ulceration, mastitis-metritis-agalactia and reproductive problems in swine (Jenkins and Hidiroglou 1972; Underwood 1977). Clinical symptoms

of Se deficiency in sheep include embryonic death, peridontal disease and scouring. In cattle Se/Vit E deficiency conditions arise as skeletal myopathy (paralytic anemia), placental retention, metritis, cystic ovaries and mastitis. Another Se responsive condition appearing in sheep, beef and dairy cattle is general unthriftiness, a condition of slow growth. The most widely recognized Se/Vit E responsive disorder in calves and lambs is NMD, also refered to as white muscle disease and stiff lamb disease. NMD is a degenerating disease of the skeletal and cardiac muscle most commonly affecting lambs and beef calves 1 to 3 months of age. A congenital form may also be found in animals at birth and in fetuses that have been aborted (Jenkins and Hidiroglou 1972).

In 1974-75 Keshan disease was identified as the first human disease associated with Se deficiency (Yang 1985). Keshan disease is a cardiomyopathy affecting primarily young peasant women and children living in mountainous and rural areas of a region extending from northeast to southwest China (Levander 1987). Selenium provided orally as sodium selenate is now used on a large scale in China as a preventative measure against the disease. Not all features of the disease are explained solely on the basis of Se deficiency and it appears other factors are involved (Yang 1985). Another disease possibly associated with Se deficiency is Kashin-Beck disease. This is a disabling polyarticular degenerative joint disease that occurs in northern China, North Korea and eastern Siberia (Levander 1987). Most

nutrient solutions administered parenterally are very low in Se (Levander 1987). A Se responsive condition in a patient receiving total parenteral nutrition has been described by Van Rij et al. (1979). The patient developed muscular discomfort in the quadricep and hamstring muscles which was alleviated by Se supplementation.

In animals and man exhibiting Se responsive conditions subnormal tissue and fluid Se levels and glutathione peroxidase (GSHPx) (glutathione: hydrogen-peroxide oxidoreductase, EC 1.11.1.9) activities are present along with a number of other biochemical changes.

It is important to recognize and distinguish between the various chemical forms of Se for the discussion of the nutritional and metabolic aspects of this element. The chemical forms of interest include: elemental Se, inorganic Se such as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), selenoamino acids such as selenomethionine (Se-Met) and selenocysteine (Se-Cys) and other organic selenocompounds. Metabolic similarities do exist but there are also some important differences between these chemical forms.

Rumen microorganisms

Se content of rumen microorganisms

The Se concentration and in vitro Se metabolism of rumen microorganisms are influenced by the previous diet of the host animal. Whanger et al. (1978) reported a variable Se

concentration ranging from 0.040 - 1.90 mg kg^{-1} in rumen microorganisms isolated from sheep on various dietary regimes. In radioisotopic studies the incorporation of ^{75}Se into bacteria in vitro was inversely proportional to the previous dietary intake of Se by the host animal (Hidiroglou et al. 1968). Whanger et al. (1978) reported a sulfur deficiency in a purified diet fed to sheep decreased the Se and nitrogen concentration of rumen microbes. Changes in microbial populations have also been observed following changes in dietary Se supplementation of a purified diet low in Se and Vit E (Hidiroglou et al. 1968).

Metabolism of Se by rumen microorganisms in vitro

Little is known of the products formed following bacterial incorporation of selenocompounds. Paulson et al. (1968) reported that 68.0% of the radioactivity added as [^{75}Se]Se-Met was found in the TCA-insoluble fraction. Radioactivity in the TCA-insoluble fraction was assumed to indicate radioactive Se associated with the bacterial protein fraction. Following a 3-h incubation period of rumen microorganisms with [^{75}Se]Se-Met, approximately 60% of the radioactivity incorported into the TCA-insoluble fraction was identified by paper chromatography and ion-exchange chromatography as [^{75}Se]Se-Met (Paulson et al. 1968). Hidiroglou et al. (1974) reported that rumen bacteria metabolized [^{75}Se]Se-Met to [^{75}Se]Se-cystine and incorporated both selenoamino acids into bacterial protein.

Paper chromatography of hydrolyzed rumen bacterial protein following a 24-h incubation of rumen microbes with [^{75}Se]SeO_3^{2-}

revealed radioactivity co-chromatographing with Se-Met (Hidiroglou et al. 1968). In addition, smaller amounts of ^{75}Se were found associated with taurine, homocystine and selenocystine. Identical fractionation patterns of ^{75}Se following incubation of rumen microbes with $[^{75}Se]SeO_3^{2-}$ and $[^{75}Se]SeO_4^{2-}$ indicated that selenate was reduced to selenite by rumen microorganisms (Paulson et al. 1968). Thus, rumen microorganisms incubated in vitro with [^{75}Se]Se-Met, $[^{75}Se]SeO_3^{2-}$ and $[^{75}Se]SeO_4^{2-}$ metabolize these chemical forms of Se and incorporate them into microbial protein (Hidiroglou et al. 1968, 1974).

Metabolism of Se in the rumen

Se administered to the rumen becomes quickly associated with the bacterial fraction. In sheep dosed intraruminally with [^{75}Se]Se-Met, 50% of the label in the rumen liquor was in the bacterial fraction 6 h after dosing (Hidiroglou et al. 1974). Approximately 66% of the label in the bacterial fraction was protein bound. Radiolabelled selenocompounds identified in the bacterial protein fraction 2 h following intraruminal administration of [^{75}Se]Se-Met included Se-Met, selenocystine and elemental Se. Unidentifed compounds constituted 40 - 50% of the radioactivity (Hidiroglou et al. 1974)

Inorganic Se is also rapidly metabolized by rumen bacteria. One hour following the intraruminal administration of $[^{75}Se]SeO_3^{2-}$, 30% of the rumen liquor activity was bound to bacterial protein (Hidiroglou et al. 1968). The highest level of

radioactivity associated with the protein fraction was 71% at 4 h. Chromatographic separation of hydrolyzed bacterial protein revealed patterns similar to that found after in vitro studies, with most of the radioactivity identified as Se-Met and smaller amounts of the radioactivity associated with selenocystine, homocystine and taurine (Hidiroglou et al. 1968). The findings obtained both in vitro and in vivo indicate rumen bacteria are capable of metabolizing inorganic Se to organic Se compounds. Metabolism of dietary Se by rumen microorganisms will thus influence the chemical form of Se available to the host animal.

Absorption

Site of Se transport across the gastrointestinal tract

The everted-gut sac technique has been used in in vitro investigations to compare the rate of absorption of various selenocompounds and to elucidate the mechanisms of transport across the intestinal cell. McConnell and Cho (1965) using everted intestinal sacs of the hamster demonstrated the rate of transport of Se-Met was highest in the distal jejunum, intermediate in the terminal ileum and lowest in the proximal jejunum and proximal ileum. In sheep, Hidiroglou and Jenkins (1973b) reported Se-Met was absorbed primarily from the mid jejunum. Radioisotopic studies showed only small amounts of [^{75}Se]Se-Met were transported across the rumen wall into the blood (Hidiroglou and Jenkins 1973a). A differing pattern of results were observed in chicks. Humaloja and Mykkanen (1986)

used an in vivo ligated intestinal loop procedure to study the absorption of labelled compounds from the different gastrointestinal segments of the chick and found a more efficient transfer of [^{75}Se]Se-Met from the duodenal segments than from the more distal segments. The variation in results with regards to the site of transport of Se-Met in the various sections of the small intestine may reflect differences in animal species or may have arisen from the experimental procedures.

Absorption of SeO_3^{2-} from the small intestine occurs primarily in the duodenum with slightly smaller amounts absorbed from the jejunum and ileum (Whanger et al. 1976; Humaloja and Mykkanen 1986) Wright and Bell (1966) studied the net absorption of Se from the intact gastrointestinal tract of ruminant and monogastric animals using [^{75}Se]SeO_3^{2-} and a non absorbable marker, chromium oxide. Net absorption of ^{75}Se occurred from the distal four-fifths of the small intestine in both species. It was not absorbed from the rumen or abomasum of sheep nor was it absorbed from the stomach of swine. There was also no absorption occurring from the cecum or colon.

Paulson et al. (1966) reported the primary site for SeO_4^{2-} absorption in the ewe was the small intestine. Wolffram et al. (1985) using an in vivo perfusion technique in rats found SeO_4^{2-} was absorbed at decreasing rates from the ileum, proximal jejunum, cecum and colon.

Mechanisms of Se transport across the gastrointestinal tract

There is little information regarding the mechanisms of Se

absorption from the gastrointestinal tract, but there appear to be different pathways involved for the different molecular forms. In experiments using the everted intestinal tract of the hamster, McConnell and Cho (1965) demonstrated [^{75}Se]Se-Met was actively transported across the small intestine. The inhibition by Met of the transport of Se-Met and vice-versa suggested that the transport system for Se-Met was the same as that for the sulfur analogue, Met (McConnell and Cho 1965). It has been suggested that high protein diets, especially diets with high Met contents, may offer a protective effect against Se toxicity through inhibition of the intestinal absorption of Se-Met by Met (McConnell and Cho 1967).

McConnell and Cho (1965) reported SeO_3^{2-} was absorbed across the small intestine by simple diffusion. The transport of selenite and selenocystine were not inhibited by sulfite and cystine, respectively, indicating there is no common transport mechanism shared by these selenocompounds and the corresponding sulfur analogues.

Wolffram et al. (1985) found SeO_4^{2-} absorption to be concentration dependent, and concluded that SeO_4^{2-} was absorbed by a saturable carrier-mediated transport mechanism in the ileal mucosa. The absorption of SeO_4^{2-} from the ileum was not affected by a 100-fold higher concentration of SO_4^{2-}. On the other hand, Cardin and Mason (1975, cited by Wolffram et al. 1985) found SeO_4^{2-} and MoO_4^{2-} inhibited SO_4^{2-} transport by the everted sacs of rat ileum, suggesting a common mechanism for absorption of

these and other anions. This mechanism however, is probably not that important in ruminants as most of the SeO_4^{2-} is likely reduced in the rumen.

Absorption of Se in laboratory animals and man

In monogastric species Se is well absorbed from the diet. Experiments conducted with laboratory animals have established inorganic Se salts (selenite and selenate) are almost as well absorbed from the intestine as selenoamino acids. In the rat intestinal absorption of SeO_3^{2-} was only slightly less than for Se-Met (Thomson and Stewart 1973). The intestinal absorption following oral administration was estimated to be 91-93% for SeO_3^{2-} and 95-97% for Se-Met. In rats fed torula yeast diets containing 0, 0.5 or 4 mg Se kg^{-1} (as sodium selenite), 95 to 100% of the Se was absorbed.

Under most experimental conditions Se is relatively well absorbed from the gastrointestinal tract of man. In general Se-Met is more completely absorbed than SeO_3^{2-}, with Se contained in food intermediate between the two (Barbezat et al. 1984). In young women Se-Met was found to be more completely absorbed (95.5 - 97.3% of administered dose) (Griffiths et al. 1976) than SeO_3^{2-} (70, 64 and 44% of the administered dose) (Thomson and Stewart 1974). Similar results were obtained for SeO_3^{2-} absorption in young adult males with 68 and 76% of the dose absorbed (Janghorbani et al. 1982a). Estimates of true intestinal absorption of food Se by New Zealand women was 76 to 83% of intake with a mean of 79%. True intestinal absorption of food Se

was higher than apparent absorption which was 49 to 60% (mean 55%) (Stewart et al. 1978). In pregnant and non-pregnant women consuming a semi-synthetic diet with egg albumin contributing the majority of Se, apparent absorption of Se was approximately 80% (Swanson et al. 1983).

Absorption of Se in ruminants

There exist relative differences between the efficiency of Se absorption by ruminants and monogastrics. Wright and Bell (1966) reported the total net absorption of Se in sheep represented approximately 35% of the Se ingested with 85% absorbed by swine when rations containing 0.35 and 0.50 mg Se kg^{-1}, respectively, were consumed.

The lower efficiency of Se absorption by ruminant animals is attributed to the action of the rumen microoganisms and the conditions within the rumen which alter the chemical form of Se ingested and thereby influence the chemical form of Se absorbed. Inorganic salts of Se (selenite and selenate) are likely reduced to insoluble forms such as elemental Se or insoluble metal selenides (Butler and Peterson 1961; Cousins and Carney 1961; Peterson and Spedding 1963) and thereby may be made less available for absorption than organic forms of Se which occur naturally in feeds. Peter et al. (1982, cited by Peter et al. 1985) reported the apparent absorption of ^{75}Se was 12% to 15% higher for [^{75}Se]Se-Met than [^{75}Se]SeO_3^{2-} in sheep of a low Se status receiving low Se diets.

Conrad (1985) reported the apparent absorption of Se in

non-lactating dairy cattle was 41% of the dietary Se intake. When Se was provided to dairy cattle from natural feedstuffs its apparent absorption was greatest at dietary calcium levels of 0.8% of dry matter intake. Amounts of dietary calcium less or greater than 0.8% resulted in a reduction of the apparent Se absorption (Harrison and Conrad 1984b). Quantitative information on the true absorption of Se in ruminants is still lacking.

Excretion

Selenium is excreted in feces, urine and expired air. The major route of excretion is a function of the animal species, the Se status of the animal, the mode of Se administration and the nature of the diet. In non-ruminant animals most dietary Se is excreted in urine. In ruminant animals the primary route of excretion is via the feces when dietary levels of Se are low to adequate. As dietary Se levels increase the urinary route of excretion may equal or exceed fecal Se excretion (Butler and Peterson 1961; Cousins and Cairney 1961; Lopez et al. 1969). In non-lactating dairy cattle consuming 400 - 3100 μg Se d^{-1}, 50 - 83% of the daily selenium intake was excreted in the feces and 7 - 14% in the urine (Harrison and Conrad 1984a).

Lopez et al. (1969) examined the excretory patterns of ^{75}Se after oral and intravenous dosing in lambs with varying levels of Se intake. The major factor affecting the fecal loss of ^{75}Se was the route of isotope administration, with much higher quantities of Se excreted in feces following oral admistration (Fig. 1).

Roughly equal quantities of the radioisotope were excreted in the feces in all groups dosed intravenously, indicating a small but constant endogenous secretion independent of dietary Se intake. The dietary Se levels were reflected in urinary and volatile Se excretion. Urinary and respiratory excretion of ^{75}Se increased with increasing dietary Se level, in particular when the isotope was intravenously administered (Fig. 1).

The pattern of ^{75}Se excretion is also influenced by the chemical form of the Se administered depending on the route of isotope administration. Hidiroglou and Jenkins (1972) report no significant differences in total radioactivity in urine or feces when radioselenium was administered orally as organic (Se-Met and Se-Cys) or inorganic (SeO_3^{2-} and SeO_4^{2-}) forms to sheep fed a low Se diet (0.02 mg kg^{-1}). There was however, greater radioactivity excreted in the urine following intraabomasal administration of radioselenate than following intraabomasal administration of the other chemical forms. Symonds et al. (1981a) also found diffences in excretory patterns of ^{75}Se following intravenous administration of radiolabelled SeO_4^{2-} and SeO_3^{2-}. More of the ^{75}Se injected as SeO_4^{2-} was excreted in feces and urine during the first 24 h after dosing than of the ^{75}Se injected as SeO_3^{2-}. The cumulative excretion of ^{75}Se from these two chemical forms in urine 14 d after intravenous dosing was equal to 10% of the initial dose. The cumulative excretion of ^{75}Se from $[^{75}Se]SeO_4^{2-}$ and $[^{75}Se]SeO_3^{2-}$ in feces was 17% and 9.5% of the dose respectively.

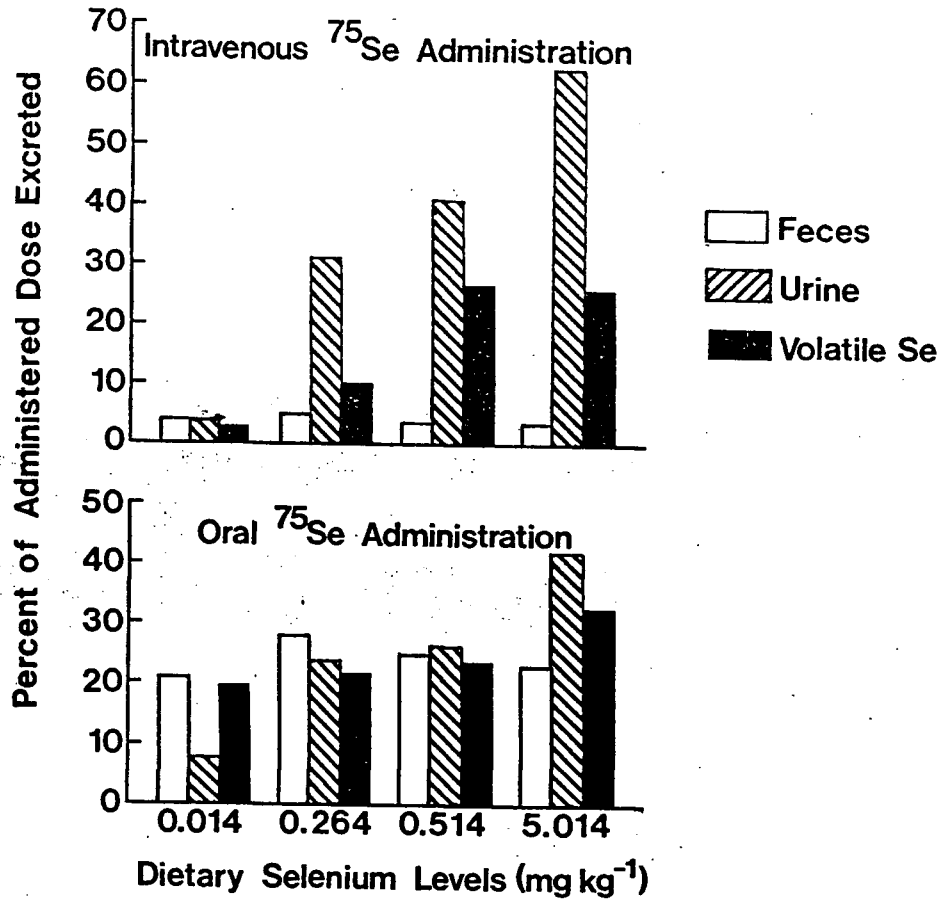


Fig. 1. Excretion of ^{75}Se during a 12-day period following oral and intravenous administration of the isotope by lambs with varying selenium intakes (Source, Lopez et al. 1969)

Selenium in the feces of ruminants includes largely Se which is unabsorbed from the diet and which has probably undergone reduction in the rumen to unavailable forms such as elemental Se and metal selenides. Selenium is secreted in bile, pancreatic and other gastrointestinal secretions all of which may not be completely reabsorbed and thus also contribute to fecal Se (Dejneka et al. 1979; Symonds et al. 1981b; Langlands et al. 1986).

Trimethyselenonium ion (TMSe) [$(CH_3)_3Se^+$] is the most well-characterized metabolite excreted in the urine (Palmer et al. 1970). In rats fed or injected with relatively high levels of SeO_4^{2-}, Se-Met, Se-cystine, Se-methylselenocysteine and seleniferous wheat, 20 - 50% of the urinary Se was identified as TMSe (Palmer et al. 1970). A second major urinary metabolite referred to as U-2 accounted for 11 - 28% of the total urinary Se. There is some controversy as to the importance of TMSe as a urinary metabolite at lower levels of Se intake. Palmer et al. (1969) found TMSe to be a major metabolite accounting for 40% of urinary Se from SeO_3^{2-} at low physiological doses. In contrast, Nahapetian et al. (1983) reported TMSe was a major urinary metabolite for near toxic doses of SeO_3^{2-} and selenoamino acids but was only a minor metabolite at low doses.

The major volatile form of Se in respiratory gases is dimethyl selenide (DMSe) [$(CH_3)_2Se$)]. Expiration of Se by animals with physiological intakes of Se does not reach significant quantities (Ganther et al. 1966). Handreck and

Godwin (1970) reported only 1% of the ^{75}Se excreted in ewes from ^{75}Se labelled rumen pellets (elemental Se) appeared in expired air. At toxic dietary Se intakes the exhalation of Se becomes an important route of excretion. In rats up to 62% of a large dose of Se was expired (McConnell and Roth 1966). The expiration of Se may also be influenced by other factors. In the rat high dietary Met and protein levels increased the expiration of Se (Ganther et al. 1966).

Lopez et al. (1969) found the formation of ^{75}Se labelled volatile compounds by sheep on a low Se diet was greater following oral administration of the dose than following intravenous administration, and suggested there was formation of volatile or gaseous Se products within the rumen.

Distribution in tissues and fluids

The predominant chemical form of Se in animal tissues is Se-Cys. In the rat 80 to 85% of the total body Se was in protein in the form of Se-Cys (Hawkes et al. 1985; Tappel 1987). Of the total body Se in the rat, one third was present as the selenoenzyme GSHPx (Tappel 1987).

Selenium is present throughout the animal body at varying concentrations depending on the cell, tissue and fluid (Underwood 1977). Listed in Table 1 are typical tissue Se concentrations for cattle of adequate Se status. The highest concentration of Se is found in the kidney with high levels also found in the liver and glandular tissues, especially in the pancreas and

adrenal glands. Intermediate levels of Se are found in the intestine, lung and cardiac muscle and low levels in the skeletal muscle, bones and blood. Even lower Se concentrations are found in the adipose tissue. In the whole blood of dairy cattle the cellular component contains approximately 73% of the total Se with the remainder in plasma (Scholz and Hutchinson 1979).

Grace and Watkinson (1985) estimated the total body Se in a 50-kg sheep with 2.9 kg fleece when maintained on a low Se diet (0.042 mg kg^{-1}) was 1.45 mg. The muscle, digestive tract, bone, kidney and liver contained respectively 40%, 12%, 10%, 7%, and 6% of the total body Se.

Tissue Se levels reflect the dietary intake of Se over a wide range. Thompson et al. (1981) monitored the response of several body components to changes in dietary Se concentration in calves transferred between low and high Se pastures. Liver and plasma Se concentrations responded most rapidly. It was concluded liver and plasma Se provided the best indicator of the current Se status of cattle. Table 2 lists tissue Se levels in liver, serum, kidney and muscle which may serve as indicators for the diagnosis of Se deficiency.

Retention of ^{75}Se by tissues and fluids

The retention of ^{75}Se by the tissues is influenced by the Se levels of the diet. In almost all tissues, percentage retention of ^{75}Se decreased with increasing dietary Se level (Lopez et al. 1969; Kincaid et al. 1977). In calves fed a practical diet containing 0.3 mg kg^{-1} of natural Se with 0.0 (control), 0.1, and

Table 1. Selenium concentration of tissues and fluids in cattle of adequate selenium status

Tissue	Selenium concentration ($\mu g\ g^{-1}$ DM)
liver	0.800 - 1.750
kidney	1.370 - 2.700
lung	0.815
heart	0.733 - 0.770
skeletal muscle	0.208 - 0.583
pancreas	1.300
spleen	0.940
adrenal glands	1.405 - 1.576
thymus	0.613
brain	0.594
uterus	0.149 †
ovary	0.230 †
testes	1.585
adipose tissue	0.031 - 0.043
bone	0.100
hair	0.400 - 1.340
whole blood	0.110 - 0.194 ‡
plasma	0.034 - 0.112 ‡

† $\mu g\ g^{-1}$ wet weight

‡ $\mu g\ mL^{-1}$

(Compiled from Perry et al. 1976; Kincaid et al. 1977; Ullrey et al. 1977; Doyle 1979; Puls 1981; and Scholz et al. 1981a)

Table 2. Selenium concentration in tissues indicating marginal and deficient selenium status in cattle

	Liver $\mu g\ g^{-1}$ †	Kidney(cortex) $\mu g\ g^{-1}$ †	Muscle $\mu g\ g^{-1}$ †	Serum $\mu g\ mL^{-1}$
Marginal	0.420-0.875	1.40-3.50	0.175-0.245	0.020-0.040
Deficient	0.07-0.595	0.630-1.40	0.035-0.175	0.002-0.008

† Dry matter basis.
(Source, Puls 1981).

1.0 mg kg^{-1} supplemental Se (as selenite) the specific activity in the kidney decreased 29% and 69% respectively in the Se supplemented groups 48 h after oral dosing of ^{75}Se. Selenium specific activities were also reduced by supplemental Se in the heart, liver, and blood but not significantly in the muscle or pancreas (Kincaid et al. 1977).

There is a wide range of ^{75}Se retention in tissues and fluids following the administration of the radioisotope (Lopez et al. 1969; Handreck and Godwin 1970; Dejneka et al. 1979; Scholz et al. 1981a). Based on ^{75}Se concentration the highest retention is found in the kidney cortex followed by in descending order: kidney medulla, testes, liver, spleen, lung and heart. High levels are also retained in the glandular tissue in particular by the pancreas and the pituitary, pineal, adrenal and salivary glands. Lower levels of ^{75}Se are retained in the smooth muscle of the gastrointestinal tract, with concentrations in the small intestine higher than in the four stomach parts, large intestine and cecum. The lowest level of radioactivity is measured in skeletal muscles, hide, bone, adipose tissue and parts of the eye.

The tissue retention of ^{75}Se, in addition to being influenced by the Se level in the diet, is also influenced by the chemical form of the element and its route of administration. In sheep there is a greater retention of ^{75}Se by the tissues following intravenous (Lopez et al. 1969) or abomasal (Hidiroglou and Jenkins 1972) administration than following oral

administration of the radioisotope.

Hidiroglou and Jenkins (1972) administered [^{75}Se]Se-Met and [^{75}Se]Se-cystine to the abomasum of sheep and found higher levels of radioactivity in the kidney, liver, and heart in those animals administered the former [^{75}Se]selenoamino acid. These differences were not found following the administration of the selenoamino acids to the rumen. Differences in the main site of radioactivity incorporation also exist between these two selenoamino acids when administered to the abomasum. In animals receiving [^{75}Se]Se-Met the highest level of radioactivity was in the pancreas whereas in animals receiving [^{75}Se]Se-cystine the highest level was in the kidney.

When [^{75}Se]SeO_3^{2-} and [^{75}Se]SeO_4^{2-} were administered to the abomasum of sheep, the latter resulted in higher radioactivity in tissue of the rumen, omasum, abomasum, duodenum, cecum, liver, and pancreas (Hidiroglou and Jenkins 1972). The tissue retention of ^{75}Se from these two inorganic Se sources were not different when they were administered to the rumen.

A comparison of the tissue retention of ^{75}Se from organic and inorganic sources yields differing results depending on the tissue examined. Peter et al. (1985) reported the specific activity and retention of ^{75}Se in the heart, lung, spleen, kidneys, whole blood and plasma to be similar in sheep administered [^{75}Se]Se-Met and [^{75}Se]SeO_3^{2-} by intravenous or abomasal routes. In contrast, there was a significantly higher specific activity and retention in muscle tissue of animals

receiving [^{75}Se]Se-Met by either route of administration. Studies in rats have also demonstrated a greater deposition of ^{75}Se in the muscle tissue following oral (Cary et al. 1973) and intraperitoneal (Beilstein and Whanger 1986) administration of [^{75}Se]Se-Met compared to [^{75}Se]SeO_3^{2-}. No differences were found for the deposition of ^{75}Se in the liver, testes, erythrocytes, hair and skin.

Despite similarities reported for certain tissue ^{75}Se activity levels following [^{75}Se]Se-Met and [^{75}Se]SeO_3^{2-} administration, a closer look at the predominant chemical form of ^{75}Se indicates some differences in their intermediary metabolism. Beilstein and Whanger (1986) reported the predominant form of ^{75}Se in erythrocyte protein lysate was Se-Cys in rats injected with [^{75}Se]SeO_3^{2-}. In the rats injected with [^{75}Se]Se-Met two unidentifiable compounds were recovered. In hemoglobin [^{75}Se]Se-Met was identified from rats injected with [^{75}Se]Se-Met but not from rats injected with [^{75}Se]SeO_3^{2-}. In acid hydrolysates of liver, ^{75}Se was recovered primarily as [^{75}Se]Se-Cys from rats injected with [^{75}Se]SeO_3^{2-}. In animals injected with [^{75}Se]Se-Met, ^{75}Se in the liver was present initially as [^{75}Se]Se-Met but after 5 d the majority of ^{75}Se was as [^{75}Se]Se-Cys (Beilstein and Whanger 1986).

Whole body turnover of Se

The whole body turnover of Se in lambs was described by two first order processes with differing rate constants (Lopez et al. 1969). The initial slope covering a 48-hr period was greater

than the final slope. As the dietary Se intake of the animals increased so did the final slope and thus the effective half-life of ^{75}Se decreased. This described the pattern following oral and intravenous ^{75}Se administration. The initial slope describing the first component of ^{75}Se clearance was considered to represent excretion of "unequilibrated" Se in animals given ^{75}Se orally. It appeared to consist of unabsorbed ^{75}Se as well as absorbed ^{75}Se excreted in the urine, expired air or via the gastrointestinal tract with little or no equilibration with the main Se pools of the body. The second component appeared to represent relatively slow turnover of Se involved in essential metabolic functions or storage.

In dairy cows administered $[^{75}Se]SeO_3^{2-}$ or $[^{75}Se]SeO_4^{2-}$ intravenously or ^{75}Se-labelled barley orally, the whole body turnover was also described by two exponential components (Symonds et al. 1981a). In animals where the first component of clearance was measurable, it was calculated to equal 1 - 1.9 days. The second component of clearance was 60.7 ± 3.9 days for all animals. Similaries in the response of the second component suggested that intravenously administered inorganic Se and orally administered organic Se were incorporated into the same metabolically active pool (Symonds et al. 1981a).

Plasma Se turnover

The decay of radioactivity in plasma after intravenous injection of $[^{75}Se]SeO_3^{2-}$ occurs in four phases (McMurray and Davidson 1985). Immediately following injection ^{75}Se is rapidly

taken up by the erythrocytes. The Se is modified by the erythrocyte and excreted as possibly hydrogen selenide (H_2Se) or another reduction product of selenodiglutathione (GS-Se-SG) (Sandholm 1973; Gasiewicz and Smith 1978) and is transferred to other tissues. The first phase represents the decay of ^{75}Se bound to plasma proteins. Symonds et al. (1981a) demonstrated the uptake of ^{75}Se by the liver of the cow was concomitant with the first phase of plasma radioactive decay. During the second phase there was an increase in the ^{75}Se activity in the plasma due to the release of protein bound ^{75}Se from the liver (Symonds et al. 1981a). There was a shift of the protein bound ^{75}Se in plasma from albumin to the alpha and gamma globulins which was also attributed to the action of the liver (Symonds et al. 1981a). The last two phases represent the slow biphasic disappearance of various selenoproteins from the plasma (McMurray and Davidson 1985). Up to twenty different selenoproteins in plasma have been separated (Davidson and McMurray 1987).

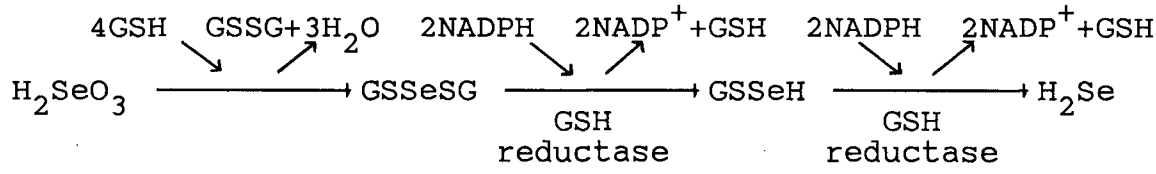
The pattern of clearance and reappearance of radioactivity of ^{75}Se in plasma is dependent on the form in which Se is administered. Radioactivity from the injection of $[^{75}Se]SeO_4^{2-}$ was cleared from the systemic circulation at a lower rate and was not released as rapidly back into the circulation as when ^{75}Se was injected in the form of SeO_3^{2-}, suggesting SeO_3^{2-} is more readily metabolized than SeO_4^{2-} (Symonds et al. 1981a).

Intermediary metabolism of Se

The chemistry of Se resembles sulfur (S) and the metabolism of Se and S in animals is similar under some circumstances, however, there are important biochemical differences between them. In both ruminants and non-ruminants inorganic forms of Se (selenate and selenite) undergo reduction, in contrast to the inorganic forms of S (sulphide and sulphite) which undergo oxidation (NAS-NRC 1983).

The proposed mechanism of SeO_4^{2-} reduction involves its activation by ATP and ATP sulfurylase to form adenosine-5'-selenophosphate which subsequently undergoes non-enzymatic cleavage catalyzed by glutathione (GSH) to form thioselenic acid ($GSSeO_3^-$) and then SeO_3^{2-} (Ganther 1984).

Selenite is reduced to H_2Se by the glutathione reductase pathway in the cytosol of the cell (Combs and Combs 1984):



Selenite first reacts non-enzymatically with glutathione followed by catalytic reduction by glutathione reductase (EC 1.6.4.2) with NADPH to yield H_2Se (Ganther 1984). This is analogous to the reduction of sulfate and sulfite to hydrogen sulfide in plant and microbial systems (Wilson and Bandurski 1958; Dilworth and Bandurski 1977). The SeO_3^{2-} taken up by erythrocytes is probably reduced by this mechanism and released from the cell as H_2Se.

At physiological pH hydrogen selenide exists as HSe^- and plays an important central role in Se metabolism (Fig. 2). It may be oxidized to elemental Se, incorporated into or interact with plasma and cellular proteins or become methylated. Hydrogen selenide is methylated in the cytosolic and microsomal fractions with methyl groups provided by S-adenosylmethionine. Selenium is excreted as methylated compounds, the best known being dimethyl selenide and the trimethylselenonium ion. DMSe is an intermediate in the pathway for the production of TMSe. The methylation of DMSe to TMSe is a rate limiting step, thus when a large dose of Se is presented to the excretory pathway DMSe accumulates and is exhaled (Palmer et al. 1969). Ganther (1987) has proposed the third methyl group of TMSe might be derived from a carbon chain rather than through the methylation of DMSe. This offered an explanation as to why the administration of arsenite to animals had no effect on TMSe formation but yet inhibited the synthesis of DMSe (Ganther 1987).

Selenomethionine is believed to follow the metabolic pathways of methionine. Selenide may be released from Se-Met by transulfuration producing Se-Cys or by transamination forming methylselenol. Selenocysteine is produced by reduction of selenocystine in a manner analagous to the reduction of cystine. Esaki et al. (1982) have identified an enzyme, selenocysteine-β-lyase which specifically catalyzes the decomposition of Se-Cys producing alanine and H_2Se.

The mechanism of Se incorporation into the Se-Cys of GSHPx

or other selenoproteins has not yet been determined. There are two proposed mechanisms, translational and post-translational, in both of which H_2Se is an intermediate. Biosynthesis of Se-Cys may arise by transfer of H_2Se to an acceptor such as o-acetyl serine. Incorporation of Se-Cys into selenoproteins could then occur translationally through a tRNA specific for Se-Cys (Hawkes et al. 1982). On the other hand, Sunde and Hoekstra (1980) believe Se-Cys is formed post-translationally by insertion of Se into an amino acid residue, possibly serine and/or cysteine present in the appropriate position of the peptide backbone.

Hydrogen selenide may also form complexes with electrophilic metabolites (X^+, i.e. metals) which may then undergo methylation thereby reducing the toxicity and/or biological availability of the components involved (Ganther 1984).

Glutathione Peroxidase

Glutathione peroxidase was shown by Rotruck et al. (1973) to be a Se-containing enzyme and is the only well-characterized selenoenzyme in higher animals at this time. It is a homologous tetramer of molecular weight 75,000 to 98,000, depending on tissue and animal species (Combs and Combs 1984). Each subunit of GSHPx contains one Se atom (Epp et al. 1983) in the form of Se-Cys (Forstrom et al. 1978). The number of active sites per tetramer has not been determined. In contrast to other peroxidases, GSHPx contains no heme or flavin moeity. The distribution and activity of GSHPx varies considerably among

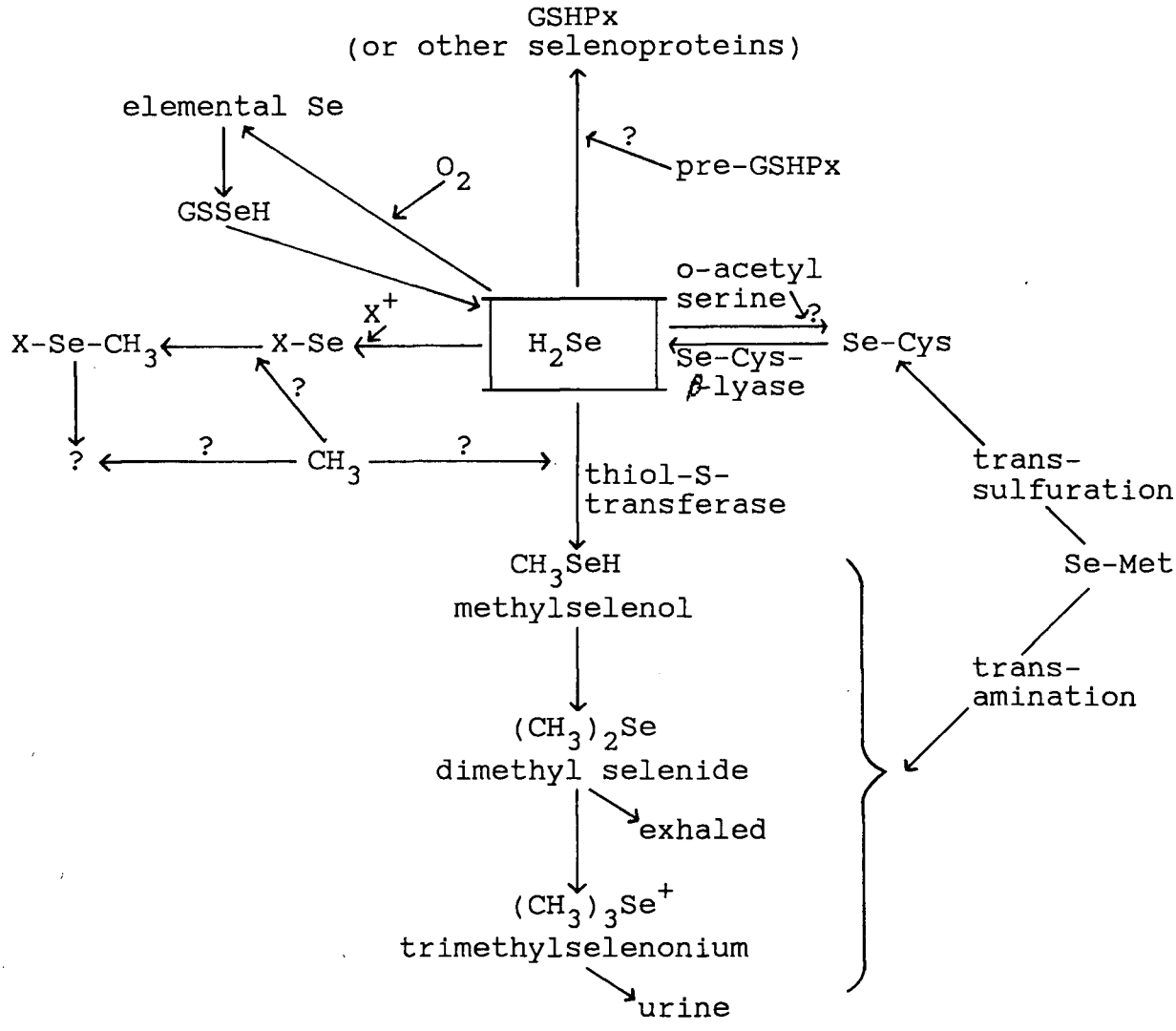


Fig. 2. Selenide metabolism

tissues and animal species. In tissues of the calf, the highest level of GSHPx activity was in the erythrocytes followed in descending order by the testes, kidneys, adrenal glands, heart, lung and liver. The lowest level of activity was in brain, skeletal muscle, adipose tissue and blood plasma (Scholz et al. 1981a). In contrast, the highest GSHPx activity in the rat was in the hepatic tissue followed by the erythrocytes. Lower levels were found in kidney, adrenal glands, heart, lung and testes (Lawrence et al. 1974). High levels of GSHPx activity were also found in the cells with phagocytic activity (Scholz et al. 1981a).

The majority of GSHPx activity in whole blood is associated with the cellular fraction with only about 1% associated with the blood plasma (Scholz and Hutchinson 1979). GSHPx accounts for approximately 75% of the total Se in erythrocytes of sheep (Oh et al. 1974), 100% in rats and 10% in humans (Beilstein and Whanger 1987). In the erythrocyte of humans the majority of Se is associated with hemoglobin (Beilstein and Whanger 1987).

Tissue activities of GSHPx vary directly with the level of dietary Se when supplemented at low levels to low Se diets. The activities in most tissues were reported by Oh et al. (1976a,b) to plateau at dietary concentrations of about 0.1 mg Se kg^{-1}, while tissue Se concentrations continued to rise above that dietary Se level. Moksnes and Norkeim (1983) reported the activity of GSHPx in tissues of lambs continued to increase until dietary Se levels reached 0.23 mg kg^{-1}. Above this level, GSHPx

activity in the tissues approached a plateau with the exception of blood GSHPx. The disparity between the activity of GSHPx and the concentration of Se in tissues in animals consuming Se adequate diets is the result of non-specific incorporation of selenium into tissue proteins which becomes more quantitatively significant with increasing Se intake (Combs and Combs 1984).

A nutritional deficiency of Se results in a decrease in tissue GSHPx activity. Plasma GSHPx activity responds rapidly to changes in dietary Se concentration and has been suggested as an indicator for assessment of Se status (Thompson et al. 1981). The value of plasma GSHPx activity for assessing Se status, is however, questionable due to its unknown origin and functional metabolic significance. In addition, bovine plasma GSHPx does not correlate well with plasma Se concentration (Scholz and Hutchinson 1979). Hepatic GSHPx has also been suggested as an indicator of short term Se status in some animal species (Scholz and Hutchinson 1979).

The activity of GSHPx in eythrocytes is highly correlated with whole blood Se over a wide range of blood Se values in sheep and cattle (Wilson and Judson 1976; Scholz and Hutchinson 1979; Thompson et al. 1981). Se is incorporated into erythrocyte GSHPx during erythropoeisis and remains there throughout the life of the cell. The lifespan of the bovine erythrocyte in circulation is 135 to 162 days (Kaneko et al. 1971). Cells are continually being produced and removed from circulation and as a result an increase in the dietary level of Se is not immediately reflected

by an increase in erythrocyte GSHPx activity (Thompson et al. 1980, 1981). Likewise a decrease in Se intake is not immediately followed by a decrease in erythrocyte GSHPx activity. For this reason measurement of erythrocyte GSHPx is considered useful for assessment of an animal's long term Se status (Thompson et al. 1981).

Glutathione-S-transferases catalyze the conjugation of a large number of xenobiotics and endogenous toxins. One or more of these enzymes also demonstrate glutathione peroxidase activity (Prohaska and Ganther 1977) and thus are sometimes referred to as Se-independent GSHPx. They catalyze the reduction of organic hydroperoxides but not hydrogen peroxide. It is therefore important to use hydrogen peroxide as the peroxide substrate when using GSHPx assays to estimate Se status from tissues having the Se-containing GSHPx and the glutathione transferases with GSHPx activity. Tissues of the calf having both enzymes include liver, lungs, adrenal glands, testes, kidney medulla and kidney cortex. The hepatic tissue contains the highest percentage of Se-independent GSHPx activity. Tissues with only the Se-containing GSHPx include spleen, cardiac muscle, erythrocytes, brain, thymus, adipose tissue and striated muscle (Scholz et al. 1981b).

Role of glutathione peroxidase in the oxidant defence system of the cell

In biological systems oxygen radicals are produced as a normal process. Examples of such reactions occur in the terminal

oxidases of the mitochondrial electron-transport system, the microsomal cytochrome P-450 containing and cytochrome b_5-containing electron-transfer systems, and in the adrenal mitochondrial system, responsible for steroid hydroxylation that also contains cytochrome P-450 species (Diplock 1987). Should production of the oxygen metabolites go uncontrolled damage to important biological macromolecules including unsaturated phospholipids of membranes, DNA and protein may result.

The reduction of molecular dioxygen to water in biological systems occurs by a process involving the sequential addition of 4 electrons (Diplock 1985) and is summarized below:

$$O_2 \xrightarrow{e^-} O_2^{\cdot -} \qquad \text{superoxide anion}$$

$$O_2^{\cdot -} \xrightarrow{e^-} O_2^{2-} \qquad \text{peroxy anion}$$

$$O_2^{2-} \xrightarrow{H^+} HO_2^- \xrightarrow{H^+} H_2O_2 \qquad \text{hydrogen peroxide}$$

$$H_2O_2 \xrightarrow{e^-} OH^- + OH^{\cdot} \qquad \text{hydroxyl radical}$$

$$OH^{\cdot} \xrightarrow{e^-} OH^-$$

The superoxide anion radical ($O_2^{\cdot -}$)and the hydrogen peroxide (H_2O_2) are both capable of inducing peroxidation of polyunsaturated phospholipids in biological membranes. The product of greater concern is the more damaging species, the hydroxyl radical ($OH^{\cdot}$). Under circumstances leading to the increased concentration of the $O_2^{\cdot -}$ and H_2O_2 these species can

react to produce significant amounts of OH· by the reaction:

$$O_2 \cdot^- + H_2O_2 \longrightarrow O_2 + OH \cdot + OH^-$$

The production of the OH· is catalyzed by the presence of iron (II) by the Fenton reaction:

$$H_2O_2 + Fe^{2+} \longrightarrow OH \cdot + OH^- + Fe^{3+}$$

The Fe^{2+} is regenerated by the reaction:

$$O_2 \cdot^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

and:

$$H_2O_2 + Fe^{3+} \longrightarrow O_2 \cdot^- + 2H^+ + Fe^{2+}$$

The Fenton reaction may also be catalyzed by other redox-active divalent cations such as Cu^{2+}. A large portion of the Cu within biological systems however is tightly bound to protein and whether catalytic quantities of free Cu in the cell exists is questionable (Diplock 1987).

Halliwell and Gutteridge (1984, cited by Diplock 1987) report a ferryl radical (FeO_2^+) is a more likely species than the hydroxyl radical for initiating peroxidation of phospholipids in biological membranes. The ferryl radical is produced through the

interaction of Fe^{2+} and H_2O_2 by the reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow FeO_2^{+} + OH^{-}$$

Iron can also catalyze lipid peroxidation as iron complexes (Diplock 1987):

$$\text{Lipid-OOH} + (Fe^{2+}\ \text{complex}) \longrightarrow \text{Lipid-O}\cdot + OH^{-} + (Fe^{3+}\text{complex})$$

$$\text{Lipid-OOH} + (Fe^{3+}\ \text{complex}) \longrightarrow \text{Lipid-OO}\cdot + H^{+} + (Fe^{2+}\ \text{complex}).$$

Selenium, manganese, copper, zinc and Vitamin E function in a multicomponent oxidant defense system to prevent the formation of hydroxyl radicals and ferryl radicals by maintaining low levels of the reactive species (Diplock 1987). The $O_2\cdot^{-}$ is reduced to H_2O_2 in the mitochondria by a manganese containing superoxide dismutase (EC 1.15.1.1) and in the cytosolic compartment by a superoxide dismutase requiring copper and zinc for catalytic activity. The resulting H_2O_2 is in turn reduced to water in the cytosol and mitochondrial matrix catalyzed by the Se-containing GSHPx. In peroxisomes H_2O_2 is reduced to water and oxygen by the enzyme catalase (EC 1.11.1.6). Vitamin E is located largely within intracellular membranes. It functions to limit the proliferation of free radical damage by scavenging lipid peroxy radicals. It thereby terminates the propagation of membrane lipid peroxidation chain reactions limiting the area of

damage within the membrane (Diplock 1984).

Mechanism of glutathione peroxidase action

Glutathione peroxidase catalyzes the reduction of H_2O_2 using reducing equivalents derived from glutathione by the the reaction (Underwood 1977):

$$2GSH + H_2O_2 \xrightarrow{GSHPx} GSSG + 2H_2O$$

$$GSSG + 2NADPH \xrightarrow{GSH\ reductase} 2GSH + 2NADP^+$$

Selenium is required for the activity of GSHPx but the mechanism of its action remains unresolved. Under physiological conditions the enzyme is largely in the reduced state. The enzyme undergoes cyclic oxidation and reduction possibly via selenol (Enz-SeH) and selenenic acid (Enz-SeOH) by the proposed scheme (Fig. 3):

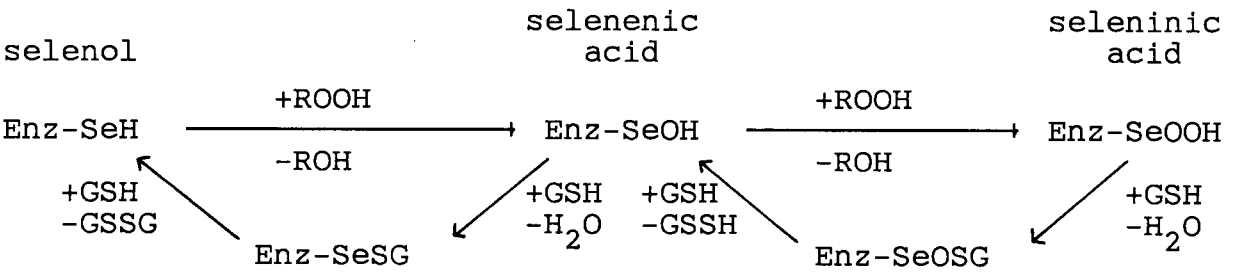


Fig. 3. Possible mechanism of glutathione peroxidase (Source, Ganther 1975)

The enzyme is oxidized by the peroxide substrate (ROOH) followed by the release of the corresponding alcohol (ROH). A selenosulfide intermediate species [Enz-SeSG] is formed during

the reduction of the enzyme by glutathione. The enzyme is further reduced by glutathione followed by the release of oxidized glutathione (GSSG). A cycle involving Enz-SeOH and seleninic acid (Enz-SeOOH) with a seleninyl-sulfide intermediate [Enz-SeOSG] has also been proposed. Iodoacetate and other alkylating agents inactivate reduced GSHPx suggesting the presence of a selenol in the reduced enzyme (Ganther 1987). It is possible however, that both cycles could operate depending on the relative concentrations of the oxidizing and reducing substrates (Ganther 1975).

The role of GSHPx in the metabolism of organic hydroperoxides in the cell is not well defined. Fatty acid hydroperoxides, the major organic hydroperoxides formed in the cells, are reduced by GSHPx when present in an unesterified form. In the cell however, fatty acid hydroperoxides exist largely in esterified phospholipids and do not seem to be available to the enzyme.

Other selenoproteins

Several non-GSHPx selenoproteins isolated from various tissues indicate there may be other important roles of Se in animals. Pedersen et al. (1972) identified a 10,000 dalton Se-containing protein in tissues of Se supplemented lambs which was absent in the heart and muscle cytosol from lambs with white muscle disease. This protein is now being referred to as G-protein.

Selenoprotein P was isolated from the plasma of the rat. It consists of two subunits: one with a molecular weight of 53,000 containing approximately 5 atoms of Se and one with a molecular weight of 35,000 containing no Se (Motsenbocker, cited by Tappel 1987). It is believed to be synthesized in the liver and may function to transport Se from the liver to extrahepatic tissues (Motsenbocker and Tappel 1982b). Beilstein et al. (1984) reported 85% of plasma Se is associated with selenoprotein P in monkey plasma. Selenocysteine is the chemical form of Se in selenoprotein P (Motsenbocker and Tappel 1982a,b) and G-protein (Beilstein et al. 1981).

Se in the testes is required for the formation of spermatozoa (Brown and Burk 1973; Wu et al. 1973) and may also function in the Leydig cells (Behne et al. 1987). Se is concentrated in the mid-piece of the sperm in a cysteine-rich structure of the outer membrane of the mitochondria. It is present as a specific selenoprotein with a molecular weight of 15,000 to 20,000 and is believed to have a structural role (Calvin et al. 1981).

Interactions of Se with Cu

Hill (1974) investigated the effect of cupric sulphate added to the diet to test its effectiveness in overcoming Se toxicity in chicks. The toxic effect of selenium dioxide (SeO_2) when included at 40 mg kg^{-1} of the diet was partially alleviated and mortality decreased by the presence of 32 and 500 mg Cu kg^{-1}.

Reaction between Cu and Se within the intestinal tract leading to the production of insoluble cupric selenides was believed responsible (Hill 1975).

Jensen (1975a) studied the effects of high levels of copper sulphate on the response of chicks to toxic levels of dietary Se. The addition of very high levels of Cu (1000 mg kg^{-1}) improved the growth rate and decreased mortality of chicks receiving 40 and 80 mg Se kg^{-1} as sodium selenite. Analysis of the liver showed significantly higher levels of Se accumulation upon the addition of Cu to the diet. The high levels of Cu reduced the availability of Se by the formation of insoluble intracellular Se compounds and to a lesser degree by interference with Se absorption.

To determine if Se deficiency could be induced in chicks by high levels of Cu in the diet, 800 or 1600 mg Cu kg^{-1} was added to a basal diet containing 0.2 mg Se kg^{-1} (Jensen 1975b). The result was high mortality and a high incidence of both exudative diathesis and muscular dystrophy. When the basal diet was supplemented with an additional 0.5 mg Se kg^{-1}, no signs of Se deficiency were observed in the chicks regardless of the level of added Cu. Van Vleet and Boon (1980) also found high levels of Cu (1500 mg kg^{-1}) added to a diet produced a high incidence of Se/Vit E deficiency in ducklings. The lesions of Se/Vit E deficiency were characterized by white areas of necrosis with or without calcification in the gizzard, intestine, skeletal muscle and heart. In contrast to the results of Jensen (1975b) and Van

Vleet and Boon (1980) which indicated that Cu induced Se deficiency, Whanger and Weswig (1978) found liver necrosis was not promoted in rats receiving Vit E and Se deficient diets when the diet contained subtoxic levels of Cu.

Gooneratne and Howell (1981) found sheep suffering from chronic Cu toxicity to have a significant increase in Se concentration and GSHPx activity in the liver. White et al. (1979, cited by Gooneratne and Howell 1981) reported a similar effect in sheep receiving increasing dietary Cu concentrations. It was suggested the increased Se retention in the sheep was a response to tissue damage caused by Cu accumulation.

White et al. (1981) investigated the effect of Cu (10 mg kg^{-1}), Mo (10 mg kg^{-1}) and Cu + Mo (10 mg Cu kg^{-1} + 10 mg Mo kg^{-1}) on the metabolism of [^{75}Se]Se-Met administered intraruminally. There was no significant effect on [^{75}Se]Se-Met excretion or retention but there was a tendency for Cu, Mo, and Cu + Mo to decrease the total retention of Se.

In view of these studies there appears to be an interaction within the tissues between Se and Cu. The effect of Cu on Se absorption however is not as clear. Studies suggesting a Cu-Se interaction in the gastrointestinal tract of simple stomached animals were investigating the effect of Cu on inorganic Se. The study investigating the effect of Cu on Se absorption in ruminants reported no antagonistic effect of Cu when Se was provided as an organic complex. These conflicting results may be due to species differences and/or the form of Se provided in the

diet (organic verses inorganic).

2.2. Stable isotopes as tracers for the study of mineral metabolism

The application of radioisotopes as tracers has made a major contribution to the understanding of mineral nutrition in man and animals. However, inherent with the use of radioisotopes is the serious issue of radiation exposure, particularly with the radiotracers with long biological half-lives. In metabolic studies with large animals, handling and disposing of radioactive excrement, fluids, and carcasses can present problems. A non-invasive, safe alternative to the use of radioisotopes is the use of stable isotopes. Stable isotopes of an element contain the same number of protons but different numbers of neutrons in their nuclei. Naturally occuring chemical elements are present as either mono-isotopes (single isotopes) or multi-isotopes (a mixture of several isotopes). Table 3 lists the number of stable isotopes for chemical elements of interest in nutrition. In principle the stable isotope method is applicable to elements with two or more isotopes.

The natural pattern of stable isotope occurance or "natural abundance" is expressed as atomic percentage. Stable isotope natural abundances are characteristic of the element. Selenium consists of six stable isotopes all of which are commercially available as highly enriched preparations. In contrast, there is

only one radioactive isotope of Se (^{75}Se) commonly available for labelling experiments thus limiting the scope of studies that may require simultaneous multiple labelling techniques.

The fundamental application of stable isotope tracers for metabolic studies requires an understanding of the potentials, limitations and measurement methodology. In principle, the application of stable isotopes as non-radioactive tracers is analogous to the application of radiotracers. In practice however, there are important differences between the two techniques. The availability of radioisotopes of high specific activity and the absence of a background level for most radiotracers of mineral elements in biological matrices, enables extremely small amounts of radiotracers to be administered experimentally. In contrast, stable isotopes are naturally present and their use requires their presence in excess of their natural isotopic abundance. The ability of the instrumentation to measure small amounts of excess enrichment on top of the natural abundance isotopic background will determine the degree of isotopic enrichment necessary. The quantity of the isotope administered experimentally is, therefore, dependent on its natural abundance, the precision of the isotopic analysis, and the degree of enrichment in the commercial preparation. It may be many times greater than the amount required for a similar study using radiotracers. The quantity of the enriched stable isotope can become a limitation in certain studies where the required amount adds significantly to the physiological level of

Table 3. Stable isotope composition of chemical elements of concern to mineral nutrition

Elements	Number of stable isotopes
Be, F, Na, Al, P, Mn, Co, As, I,	1
Cl, K, V, Cu, Br, Rb, Ag	2
Mg, Si, S, Ca, Cr, Fe, Ni, Zn, Se, Sr, Mo, Cd, Sn, Ba, W, Hg, Pb	3 or more

(Source, Janghorbani 1984)

the element. Such a situation may be overcome by administering the required dose over several days or, if possible, by increasing the precision of the isotope measurement.

The stable isotope technique has been used extensively for the investigation of amino acid, carbohydrate and lipid metabolism using ^{13}C, ^{15}N, and ^{2}H labelling and to a limited extent for mineral metabolism.

Measurement of stable isotopes

Methodology should be capable of absolute and relative isotopic abundance measurements in the matrices of interest with the required degree of accuracy and precision and at the levels resulting from physiological levels of intake of isotope. There are two general methods available for stable isotope measurement, neutron activation analysis (NAA) and mass spectrometry (MS).

NAA is based on the interactions of thermal neutrons with the nuclei of stable isotopes. The nuclei capture neutrons to yield radiotracer nuclei with various half-lives. The decay of these radioisotopes results in the emission of characteristic gamma radiation which is measured with a high-resolution gamma spectrometry system. To date NAA has been more widely applied to investigations of mineral nutrition and metabolism than has MS. NAA has been successfully applied for measurement of three of the stable isotopes of selenium, ^{74}Se, ^{76}Se, and ^{80}Se in such matrices as feces, plasma, red blood cells and urine of humans (Janghorbani et al. 1981). An analytical precision and accuracy

of 5-10% was reported for routine measurement of these isotopes which was considered satisfactory for experiments concerned with gastrointestinal absorption in human subjects.

MS is well established in geochemistry and related applications but its application for mineral nutrition has been limited. A lack of routine procedures and methods for trace element isotopic measurements of biological matrices has been a factor in its limited use.

Thermal ionization mass spectrometers provide high precision measurements of isotopic ratios for the majority of mineral elements. Its application to stable isotopic tracer studies is limited however, due in part to the high instrument cost, the degree of technical skill required and the slow sample throughput. Another instrument with an extremely high measurement precision is the isotope ratio mass spectrometer. This is used for gas analysis of isotopically enriched H, C, O, and N.

A method for stable isotope tracer studies involving complex matrices is GCMS. The sample components of interest must be converted to thermally stable volatile complexes. The ions measured may be produced from the entire derivatized molecule or from suitable fragment ions. Despite some successes, the application of GCMS suffers from problems related to the preparation of suitable chelates, limitations due to overlapping of minor isotopes of C, H, O in the chelate, and lower precision. Methodology for the application of GCMS for measurement of double

isotopic enrichment of Se in biological samples has been reported by Reamer and Veillon (1983).

Two newer approaches being explored for the measurement of stable isotopes are fast atom bombardment mass spectrometry (FABMS) and ICPMS. Possibly the greatest potential for precise measurement of stable isotopes over a wide range of chemical elements lies with the development of ICPMS. The technique offers rapid analysis and potential simplicity of chemical manipulations. It provides advantages over GCMS applied to metal chelates, by the avoidance of overlap of minor isotopes of the organic component.

In the traditional application of mass spectrometry, the method measures only isotope ratios. Absolute quantities of elements are determined by application of principles of isotope dilution with mass spectrometric measurement or by an independent elemental analysis technique. Isotope dilution eliminates the need for additional sample preparation usually required for elemental analysis and permits an accurate determination of the trace element content of a particular sample as well as allowing stable isotopes to be used as tracers. Isotope dilution is based on the addition to the sample of an exact known quantity of an enriched stable isotope of the element to be analyzed (referred to as an internal standard or spike isotope). Chemical processing of the sample must then render the internal standard and endogenous element in the same chemical form. Given this, the enriched internal standard isotope serves as an "ideal"

standard with incomplete recoveries affecting the analyte and the internal standard in the same manner and offers the advantage that quantitative recovery is not required. The addition of the internal standard to the sample alters the isotope abundances of the element in the sample. From the altered isotope ratio, mass of the sample, mass of the added internal standard and additional data, the concentration of the element originally present in the sample can be calculated.

The application of this technique for quantitation of an element in an isotopically enriched sample requires the element to consist of at least three isotopes, because of the need for two isotopes of unaltered natural abundance in enriched samples. This limits its application for studies involving elements with only two isotopes such as copper and for some studies involving multiple in vivo labelling. It is possible to analyze samples both before and after spiking in these cases, but this requires twice the number of analyses.

3. MATERIALS AND METHODS

The research for this project was conducted using the laboratory facilities and dairy herd at the Agriculture Canada Research Station in Agassiz, B. C. Cows of the Research Station's dairy herd normally receive injections of a commercial preparation containing Se and Vit E at the time of drying off. The animals used for the purpose of this research did not receive the injections. It was also ensured the animals had never received enriched Se stable isotopes at any time prior to the trials.

3.1. Analytical techniques

The notation used when referring to the enriched isotope preparations is, e.g. "Se-76". The enriched stable isotopes, Se-76, Se-77, Se-78 and Se-82 were purchased from Oak Ridge National Laboratory (ORNL), Oak Ridge, TN. The isotopic composition of the preparations (identified by ORNL sample no.) were supplied by ORNL (Appendix Table 1). Elemental Se (Se pellets, 99.9999%) was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Instra-analyzed hydrochloric acid (HCl) and nitric acid (HNO_3) and Ultrex grade HNO_3 were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. Magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$), AnalaR grade was from BDH Chemicals, Vancouver, B.C.; and magnesium (Mg) metal from either BDH Chemicals (as ribbon) or Morton Thiokol Inc., Alfa Products, Danvers, MA (as

grignard turnings, 99.99%). Toluene and chloroform from Caledon Laboratories, Georgetown, Ont. (distilled in glass) was redistilled before use.

All plastic and glassware was soaked in 10% HNO_3 for a minimum of 1 hour and rinsed several times with deionized water (deionized to a resistivity of 18 megaohms cm^{-1}). All water used was deionized.

Analysis for Se and Se stable isotope ratios for samples of Trial I was by GCMS and for samples of Trial II and III by ICPMS.

3.1.1. Gas chromatography mass spectrometry

Instrumentation: The instrument was a Hewlett Packard Model 5985^B quadrupole GCMS with select ion monitoring (SIM) (Department of Civil Engineering, UBC, Vancouver, B.C.). The following GCMS parameters were used:

column - DB-5, 0.32 mm ID x 25 m

splitless injection time - 0.5 min

temperature program - 50 - 270 C (1 min hold, 10 C min^{-1} ramp)

injection port temperature - 280 C

interface temperature - 280 C

ion source temperature - 200 C

SIM dwell time/mass - 20 μsec.

Derivatizing reagent: The derivatizing reagent was 4-nitro-1,2-phenylenediamine (4NPD) (Aldrich Chemical Co., Inc., Milwaukee, WI) and was prepared as described by Reamer and

Veillon (1981).

Nitric acid-magnesium nitrate ashing aid: The ashing aid was prepared by dissolving 40 g $Mg(NO_3)_2 \cdot 6H_2O$ per 100 mL HNO_3 by slight heating.

Sample preparation: Fifteen mL of ashing aid was added to samples of size 1 - 1.5 g or 5 - 10 mL in 200 mL Berzelius beakers. Quantification of Se was by the application of stable isotope dilution using Se-78 as the internal standard. To each sample 260.3 ng Se-78 (ORNL sample 199901) in 1% HNO_3 was added. Non-enriched samples of the same or similar biological matrix and reagent blanks were included with each batch of samples. The beakers were heated on a hot plate for 60 min at 105 C followed by 30 - 90 min (until samples were digested) at 115 C. Samples were taken to dryness on the hotplate then ashed for 4 h at 500 C (Isotemp Programmable Ashing Furnace Model 497, Fisher Scientific, Vancouver, B.C.). The ash was wetted with 5 mL of water, and dissolved in 15 mL HCl. Samples were boiled gently for ~1 min to reduce all Se(VI) to Se(IV). It was important for all Se to be converted to Se(IV), as the derivatizing reagent reacts only with Se in that valence state. After cooling the contents of each beaker were quantitatively transferred to separatory funnels with the aid of 20 mL water.

The hydrochloric salt of 4NPD was prepared as described by Reamer and Veillon (1981). One mL of 1% 4NPD (wt/vol) was added and samples let stand for 2 h at room temperature under subdued light. The reaction between 4NPD and Se(IV) produced the

thermally stable compound, 5-nitropiazselenol (NPS). The NPS formed was extracted into 5 mL toluene by shaking for 8 min on a mechanical shaker. The toluene extracts were separated from the aqueous phase, transferred to 16 x 150 mL test tubes and evaporated to dryness by an evapomix (Model 3-2100, Buchler Instruments, Fort Lee, NJ). The residues were dissolved in 2 x 100 μL chloroform, transferred to reacti-vials (0.3 mL capacity, Pierce Chemical Co., Rockford, IL) and dried under a stream of N_2. Samples were sealed under N_2 and refrigerated until analyses.

Analysis: Samples were taken up in toluene with gentle heating to ensure complete dissolution of the complex and injected into the GCMS. With selected ion monitoring (SIM) the MS was tuned for 225, 227 and 229 mass to charge ratio (m/z).

Accuracy and precision: The accuracy of the measurement of Se stable isotopes was determined by the analyses of two sets of double isotope calibration standards. Standards were prepared by the addition of five levels of Se-76 and Se-78 over ranges of 0.0 to 1.229 μg and 0.0 to 8.676 μg respectively, in all possible combinations to 10.055 μg of natural abundance Se (^{na}Se). Blank standards were also included.

The accuracy of the method for quantitative Se analysis was evaluated by the analyses of standard reference materials of bovine liver (1577) and orchard leaves (1571) (U.S. National Bureau of Standards (NBS), Washington, D.C.). The NBS standards were prepared as described for the samples.

Natural abundance Se standards containing ~5 μg Se were analyzed in triplicate on each day of sample analysis and the results used for calculation of the precision. The detection limit was defined as the level of enrichment above which the probability of obtaining a measure of natural abundance Se was < 0.05 for a single analysis of a sample. The dynamic range was defined as the dilution factor that a tracer in a tissue may undergo and still remain detectable based on an initial tracer enrichment equal to 10% of the natural Se.

3.1.2. Inductively coupled plasma mass spectrometry

Instrumentation: The sensitivity of the analysis for Se is enhanced many orders of magnitude when sample introduction is by hydride generation in place of nebulization. A continuous flow hydride generator described by Buckley et al. (1987) was coupled to a Perkin-Elmer model ELAN 250 ICPMS equipped with mass flow controllers on injector and auxiliary gas flows.

The instrument parameters were:

argon flow rates - plasma 2 L min^{-1}
injector 1.4 - 1.6 L min^{-1}
auxiliary 1.5 L min^{-1}

power - incident power 1.0 kWatt
reflected power 0 Watt

ion optics - B lens 40
P lens 10
E1 lens 85

S2 lens 31

detector - channel electron multiplier 3600 V

deflector - +4500 V

machine configuration - sample delay 90 s

wash delay 60 s

Hydrochloric acid concentration: The interaction between HCl concentration, Cu concentration and the intensity of the Se signal was investigated by the analyses of a series of samples containing 20 ng ^{na}Se mL^{-1} in 4 - 10 M HCl with Cu additions ranging from 0 to 400 μg mL^{-1}.

Nitric acid-magnesium ashing aid: Three g Mg was dissolved per 100 mL of HNO_3.

Sample preparation: Samples of size (max 1.5 g dry matter or 10 mL volume) containing 8 - 600 ng Se were weighed or dispensed into 200 mL Berzelius beakers. Forty ng Se-76 (ORNL sample 194802) in 1% HNO_3 and 15 mL of nitric acid-magnesium ashing aid was added to each sample. Non-enriched samples of the same biological matrix and reagent blanks were included with samples. Samples were placed on a preheated aluminum block for 30 min at a sample temperature of 75 C followed by 2 h at a sample temperature of 115 C. Samples were taken to dryness then ashed for 4 h at 500 C. After cooling the ash was wetted with 5 mL water and dissolved in 15 mL HCl. The contents of each beaker were transferred to 20 mL scintillation vials for analysis by ICPMS.

Analysis: A 1% (wt/vol) sodium borohydrate solution in 0.1 M sodium hydroxide was prepared daily for generation of volatile Se hydrides. The ICPMS was tuned to measure ion intensity at m/z 76, 77, 78 and 82. Reagent blanks were pooled and analyzed after every 3 samples to correct for instrument drift. The natural abundance samples or standards were analyzed in triplicate during sample analysis, each day.

Accuracy and precision: The accuracy and precision were evaluated as described for GCMS with any changes noted below.

Single isotope calibration standards were prepared for each of Se-76 (ORNL sample 194802), Se-77 (ORNL sample 194901) and Se-82 (ORNL sample 195201) alone, over a range of enrichment levels expected in samples. Another set of isotope calibration standards were also prepared for each isotope over the same range of enrichment to which was added the highest level of the other two Se isotopes (refered to as secondary Se isotopes). Standards were prepared by adding the appropriate quantity of Se-76 (0 - 325.1 ng), Se-77 (0 - 19.3 ng) and Se-82 (0.0 - 19.8 ng) to ~200 ng ^{na}Se. Blanks were included with the standards.

NBS standard reference materials of bovine liver (1577), bovine serum (8419), freeze dried urine (2670) and rice flour (1568) were prepared as described for samples.

The precision for the measurements of Se-76, Se-77 and Se-82 were determined from the analyses of natural abundance serum, feces and urine.

3.2. Calculation of Se stable isotope enrichment

Stable isotope enrichment was expressed as TTMP equal to 100 x the mass of the tracer divided by the mass of the tracee; i.e. tracer/tracee mass percentage. The tracer was defined as the enriched stable isotope of the element of interest: Se-76, Se-77 and Se-82. The tracee is the naturally occuring element, Se. The equations for TTMP for solution of up to three simultaneously enriched isotopes of Se were derived by Buckley (1987). The sets of simultaneous equations for two and three enriched isotopes are given below.

Reference isotope.....the isotope ion chosen as the denominator for ion intensity ratios (GCMS, 80; ICPMS, 78).

v, w, x and y.....subscripts referring to stable isotopes of an element and designating mass/charge (m/z) ratios of the isotope ion or the nominal mass of the isotope. When v is in parentheses it refers to the internal standard enriched in the indicated stable isotope. When x and y are in parentheses they refer to the tracer enriched in the indicated stable isotope. Isotope w is always the reference isotope.

n, m.....subscripts designating tracee (natural Se) or a mixture of tracer and tracee respectively.

M.....exact atomic mass for substance indicated in parentheses (e.g. $M_{(76)}$ = exact atomic mass of Se-76.

H.....ion abundance of the designated isotope for the substance indicated in parentheses (e.g. $H_{76(n)}$ = ion intensity at m/z for isotope 76 in natural Se divided by the total ion intensity for natural Se).

θ.....ion intensity ratio of the designated isotope over isotope w for the substance indicated in parentheses (e.g. $\theta_{76(m)}$ = ion intensity at m/z for isotope 76 divided by intensity at m/z for isotope w for a mixture of natural Se and tracer).

B.....mass of tracee (ng).

D.....mass of tracer (ng).

Two enriched isotopes (v and x):

$$TTMP_v = 100 \frac{M_{(v)}H_{w(n)}(jp + ur)}{M_{(n)}H_{w(v)}(us - hp)}$$

$$TTMP_x = 100 \frac{M_{(x)}H_{w(n)}(rh + js)}{M_{(n)}H_{w(x)}(us - hp)}$$

Three enriched isotopes (v, x and y):

$$TTMP_v = 100 \frac{M_{(v)}H_{w(n)}(eip + gjp + cjq + equ + gru - cir)}{M_{(n)}H_{w(v)}(gsu - aqu - cis - ghp - chq - aip)}$$

$$TTMP_x = 100 \frac{M_{(x)}H_{w(n)}(ehq + ghr + air + eis + gjs - ajq)}{M_{(n)}H_{w(x)}(gsu - aqu - cis - ghp - chq - aip)}$$

$$TTMP_y = 100 \frac{M_{(y)}H_{w(n)}(ajp + aru + chr + cjs + esu - ehp)}{M_{(n)}H_{w(y)}(gsu - aqu - cis - ghp - chq - aip)}$$

Where:

$a = \theta_{y(v)} - \theta_{y(m)}$ $\quad$ $c = \theta_{y(x)} - \theta_{y(m)}$

$e = \theta_{y(n)} - \theta_{y(m)}$ $\quad$ $g = \theta_{y(m)} - \theta_{y(y)}$

$h = \theta_{x(v)} - \theta_{x(m)}$ $\quad$ $i = \theta_{x(y)} - \theta_{x(m)}$

$j = \theta_{x(n)} - \theta_{x(m)}$ $\quad$ $p = \theta_{v(x)} - \theta_{v(m)}$

$q = \theta_{v(y)} - \theta_{v(m)}$ $\quad$ $r = \theta_{v(n)} - \theta_{v(m)}$

$s = \theta_{v(m)} - \theta_{v(v)}$ $\quad$ $u = \theta_{x(m)} - \theta_{x(x)}$

$\theta_{v(m)}$, $\theta_{v(n)}$, $\theta_{x(m)}$, $\theta_{x(n)}$, $\theta_{y(m)}$ and $\theta'_{y(n)}$ were obtained from mass spectrometric analysis after subtraction of reagent blank ion intensities. The remaining factors in the equations were calculated from specifications supplied with the purchased isotopes (Appendix Table 1) and from the Handbook of Chemistry and Physics (1970).

The organic component of the NPS ion contains 6 carbon atoms (C), 3 nitrogen atoms (N), 2 oxygen atoms (O) and 3 hydrogen atoms (H). Of these, 1.11% of the C atoms are ^{13}C, 0.36% of the N atoms are ^{15}N, 0.2% and 0.04% of the O atoms are ^{18}O and ^{17}O, and 0.015% of the H atoms are ^{2}H. The occurrence of these "satellite" isotopes in the organic component of the ion alters the ^{76}Se-NPS/^{80}Se-NPS and ^{78}Se-NPS/^{80}Se-NPS ratios from $^{76}Se/^{80}Se$ and $^{78}Se/^{80}Se$. Based on the probabilities of occurrence of each of the satellite isotopes, the proportion of molecules (NPS) with each possible molecular mass was calculated using the elementary laws of probability (Pickup and McPherson 1976). The proportions

of the arrangements contributing to the same molecular mass were summed enabling the probability of occurrence, P_1, P_2 and P_3 in the mass spectrum for a given molecular mass with m/z, m/z+1 and m/z+2, respectively, to be determined. The probabilities of occurrence were used to adjust the ion abundances of the enriched isotope preparations and of natural Se by the equations:

$$H_{225} = P_1A_{76} + P_3A_{74}$$
$$H_{227} = P_1A_{78} + P_2A_{77} + P_3A_{76}$$
$$H_{229} = P_1A_{80} + P_3A_{78}$$

where A is the atomic abundance of the isotope indicated by the subscript. The adjusted ion abundances were used to calculate the factors in the TTMP equations which were not obtained from GCMS analysis.

The quantity of tracee (B) in a sample is calculated from the relationship:

$$TTMP_V = \frac{D_V}{B} \times 100$$

thus

$$B = \frac{D_V}{TTMP_V} \times 100$$

Knowledge of B and $TTMP_{x \text{ or } y}$ enables the quantity (ng) of tracer ($D_{x \text{ or } y}$) in the sample to be determined from the relationship:

$$TTMP_{x \text{ or } y} = \frac{D_{x \text{ or } y}}{B} \times 100$$

thus

$$D_{x \text{ or } y} = \frac{TTMP_{x \text{ or } y} \times B}{100}$$

Total selenium (ng) equals the sum of $D_{x \text{ and } y}$ and B.

3.3. Trial I

3.3.1. Animals

Two non-lactating, non-pregnant Holsteins (8219 and 7939), culled from the herd for failure to conceive (believed to be unrelated to Se nutrition) were used. The cows had been dry for about 3 months prior to the trial.

The cows were weighed on two consecutive days at the beginning and end of the trial period to obtain an estimate of their true weight. The test cows were housed in box stalls for the first 13 days. On the 14th day the cows were moved into stanchion stalls where they remained for 13 days.

3.3.2. Diet

Prior to the trial the test cows were maintained on orchardgrass pasture and received no supplemental Se. The experimental diet was low-Se orchardgrass hay. It was chopped to facilitate the weighing of feed and weighback necessary during the balance trials. The diet was offered free choice with a minimum weighback of 2 kg each day. Water was provided free choice to each animal from individual water bowls with flow meters. Cobalt-iodized salt was provided ad libitum. The test cows were adapted to the experimental diet over a 15-d period (Days 1-15). After diet adaptation, two 5-d balance periods were run consecutively, during which the the test cows were individually fed the experimental diet. The weight of the experimental diet fed and weighback, and the water intakes were recorded daily at 10:00 am.

3.3.3. Single isotope enrichment of the whole body Se pool

Preparation of Se-76 tracer dose: In a 100 mL volumetric flask 44.47 mg of the Se-76 isotope (ORNL sample 194802) was dissolved in 5 mL of HNO_3 (Ultrex) overnight. It was diluted to volume with water to yield a solution of 444.7 μg Se-76 mL^{-1}. Dissolution in HNO_3 oxidized the Se to selenious acid (H_2SeO_3), changing the valence state from 0 (elemental Se) to IV (selenite). The daily tracer dose of 4.00 mg Se-76 was prepared by diluting 9 mL of the Se-76 solution to 250 mL with water.

Administration of Se-76 tracer: The Se-76 tracer dose was administered to the rumen by stomach tube. One dose (4.00 mg Se-76) was administered to each cow each day for a total of 5 days (Days 1-5). The bottle containing the dose was thoroughly rinsed with approximately 1 L of water and the washings also administered to the rumen. Administration of the tracer took place at 9:30 am. Based on an estimated whole body Se pool equal to 70 mg and retention of 30% of the dose, the administration of 20.00 mg Se-76 was predicted to result in a whole body enrichment of Se-76 equivalent to 10% of the whole body pool size.

3.3.4. Balance periods

Two days prior to the balance periods the test cows were prepared for urine and fecal collections. A thick layer of a rubber based glue was applied surrounding the anal and vulval area of the cows. Once the glue had partially set (~1 h) a plastic mesh and another layer of glue were applied. The glue was allowed to dry overnight. The end of a rubber hose (~2.5 m long) was stretched over a wire shaped to encircle the vulva, and stitched to the plastic mesh. Urine was channelled down the hose into a covered stainless steel container. Collection boxes were positioned in the gutter behind the cows for fecal collection. Feces were transferred several times a day from the collection box into covered plastic buckets.

Two 5-d total collection balance periods were run

consecutively following the dietary adaptation (Period 1, days 15-20; Period 2, days 20-25). Complete collections of feces and urine were made every 24 h and the daily outputs of each were recorded. The feces was mechanically mixed for ten minutes. A ~500 g sample was transferred to an aluminum tray, weighed and frozen. A subsample of ~450 mL of urine was transferred to a plastic bottle, acidified with HNO_3 (Ultrex) and frozen. Three feed and water samples were collected over both periods. Water samples were acidified with HNO_3 (Ultrex).

Dry matter percentages of feed and feces samples were determined by lyophilization. Feed and feces samples were ground to pass through a 1 mm stainless steel screen. The three feed and water samples were each composited. Pooled samples of feces and urine were prepared for each of the 5-d balance periods.

Blood samples were obtained by jugular vein puncture at the beginning of each balance period and at the end of the second balance period. Fifty mL of blood was collected in 2 X 25 mL tubes without anticoagulant (Sarstedt Canada, Inc., Que.). The blood samples were allowed to clot and centrifuged at 4000 x g for 10 min to separate the serum. Serum was decanted off and frozen.

One 24-h total collection of feces and urine was made from a dry cow which had never before received enriched Se stable isotopes. This was begun at the same time as the start of the first balance trial with the test cows. These samples were to determine isotope ratios of natural abundance Se in these sample

matrices.

The feed was analyzed for protein by a modification of the Kjeldahl procedure with colorimetric determination (AOAC 1980); acid detergent fiber (Van Soest 1963); calcium, magnesium, potassium, iron, manganese, and zinc by atomic absorption spectrometry (British Columbia Ministry of Agriculture and Fisheries (BCMAF), Soil, Feed and Tissue Testing Laboratory, Kelowna, B.C.); phosphorus by colorimetry (BCMAF); and sulfur, molybdenum, and copper by ICPMS following sample decomposition with nitric and perchloric acids. Feed, water, feces, urine and serum were analyzed for total Se and Se stable isotope ratios by mass spectrometry.

3.3.5. Tissue collection

The test cows were sacrificed following completion of the second balance period. Test cow 8219 was slaughtered at Scott's Meats (Agassiz, B.C.) and cow 7939 at the Veterinary Pathology Laboratory (Abbotsford, B.C.). Samples of all major tissues and fluids of the cows were obtained (Table 4). Large samples and in some cases whole organs were collected, placed in individual plastic bags on ice and transported immediately back to the lab to be subsampled.

Stainless steel instruments used for subsampling the tissues were soaked in saturated ethylenediaminetetraacetic acid (EDTA) and rinsed with water. Where possible samples were obtained from the interior of tissues to avoid contact with benches, other body

fluids etc. Further notes on sampling individual tissues are given in Table 4. The tissues were rinsed with water and drained on ashless filter paper. Samples of 200 - 300 g fresh weight were transfered to tared specimen cups, weighed and dry matter percentage determined by lyophilization. Tissue samples were analyzed for total Se and Se stable isotope ratios by mass spectrometry.

3.4. Trial II and III

As only small numbers of animals were manageable by one person it was necessary to conduct the second phase of the experiment as two trials (Trial II and III) of six animals each.

3.4.1. Animals

Two groups of six non-lactating, pregnant Holstein cows in their second, third or fourth dry period were used. The cows were weighed on two consecutive days at the beginning and end of the trial. They were maintained within stanchion stalls for the duration of the experiment (27 days).

3.4.2. Experimental diets

The animals in each trial were randomly assigned to one of two dietary Cu treatments:

Treatment 1 - control, no supplemental Cu

Treatment 2 - 20 mg kg^{-1} DM of supplemental Cu.

Table 4. Description of tissue and fluid samples collected

Tissue	Description
liver	Individual samples were taken from left and right dorsal, left and right ventral and caudal lobes.
kidney	Cortex and medulla were separated.
spleen	
pancreas	
lung	
heart	
skeletal muscle	Samples were taken from the semimembranosus semitendinosus, biceps femoris, longissimus dorsi, superficial and deep pectoral muscles.
rumen	Epithelium and smooth muscle were separated.
reticulum	See rumen.
omasum	See rumen.
abomasum	See rumen.
small intestine	Lumen was rinsed repeatedly with water.
colon	See small intestine.
udder	
bone	Samples were taken of femur, humerus, and rib.
hide	All hair was removed with a scalpel.
uterus	Epithelium and smooth muscle were separated.
perirenal fat	
thoracic fat	
bile	Collected in tared specimen cup directly from the gall bladder.

The basal diet was cubed orchardgrass hay fortified with Se and Zn (Treatment 1 and 2) and Cu (Treatment 2). The Se (sodium selenite), Zn (zinc sulphate) and Cu (copper sulphate) were dissolved in water and mixed with molasses. The mineral-molasses mixture was sprayed on ~200 kg batches of chopped orchardgrass hay (~4% molasses wt/wt) and the hay mixed. The hay was then cubed to facilitate handling. The dietary adaptation period was 14 days.

3.4.3. Double isotope enrichment of the whole body Se pool

The administration of Se-77 (intrarumen) and Se-82 (intravenous) was predicted to achieve a total whole body enrichment of 3 - 4%. Dosing took place on the same day as the commencement of the dietary adaptation period.

Preparation and administration of Se-77: An exact mass of Se-77 (ORNL sample 194901) (Trial II, 29.881 mg; Trial III, 28.186 mg) was dissolved in 10 mL HNO_3 (Ultrex) and diluted to volume with water in a 100 mL volumetric flask. The tracer dose was prepared by diluting 16 mL of the Se-77 solution to 250 mL. One dose was administered intraruminally to each animal (See trial I, 3.3.3.). The exact mass of the isotope administered to each animal is listed in Table 5.

Preparation of Se-82 infusate: An exact mass of Se-82 (ORNL sample 195201) (Trial II, 8.718 mg; Trial III, 7.963 mg) was dissolved in 3.5 mL HNO_3 (Ultrex) and 3.5 mL water. The

solution was quantitatively transferred with 4 x 5 mL water washings to a tared 50 mL autoclavable bottle, diluted to ~30 mL water and the exact weight recorded. Capped with a teflon faced septum and aluminum seal, the solution was sterilized by autoclaving at 15 psig steam pressure for 20 min. The bottle was weighed before and after autoclaving to check for loss of solution. Using sterile technique and 5 cc disposable syringes, ~5 mL of the Se-82 solution was injected into 1-L bags of sterile saline (0.9% NaCl) labelled for each cow. The exact mass of the solution transferred to each bag was determined by weighing the syringe before and after injection. The weight of each bag of saline was recorded following the addition of Se-82 and following intravenous administration.

Intravenous infusion of Se-82: A 14 gauge needle was inserted into the jugular vein and approximately 2 feet of a teflon catheter was fed down the vein toward the heart. Using a 5 cc disposable syringe, a few mL of sterile heparinized saline was injected in the catheter then drawn back until blood appeared in the tube to test the catheter implant and remove clots. The administration of saline was begun and once all animals were connected to saline, the saline bag was switched to the Se-82 containing infusate and the infusion started. Infusions took place over a 3 - 4 h period. When approximately 50 mL of the experimental infusate remained, the bag was switched back to saline solution for 20 - 30 min to rinse the catheter tube. The mass of Se-82 infused into each animal is listed in Table 5.

Table 5. Mass of Se-77 and Se-82 administered to each cow in Trial II and III

Cow No.	Trial No.	Se-77 (194901) mg	Se-82 (195201) mg
8222	II	4.7810	1.2380
8228	II	4.7810	1.4035
8328	II	4.7810	1.3749
8022	II	4.7810	1.3793
8238	II	4.7810	1.3483
8337	II	4.7810	1.4065
8334	III	4.5098	1.2336
8227	III	4.5098	1.2174
8329	III	4.5098	1.1902
8240	III	4.5098	1.2341
8307	III	4.5098	1.1889
8331	III	4.5098	1.2411

3.4.4. Balance periods

The total output of urine was collected using indwelling catheters (size 24 FR balloon catheter, balloon size 75 mL, Rusch, Scarborough, Ont.). Catheters were inserted by a veterinarian according to the procedure of Crutchfield (1968), two days prior to the commencement of the balance periods. During these two days the cows were observed for any sign of discomfort and hematuria. The catheters remained in place for the duration of the balance periods. Twice daily, the vulval area was washed with warm external disinfectant and an antibiotic ointment applied.

Two 5-d total collection periods were run following the dietary adaptation period. The record of output for feces and urine, record of intake for feed and water and sample collection and analysis were as described for Trial I (Refer to 3.3.4.). Blood samples were collected one day prior to the trial and on the first, third and fifth day of each balance period. Blood was collected and analyzed as described for Trial I.

3.4.5. Liver biopsy

Liver biopsies were taken by a veterinarian two days after the completion of the balance periods. The biopsy instrument used was as described by Buckley et al. (1986). The preparation of the insertion site and the biopsy procedure were according to the method of Pearson and Craig (1980). To obtain approximately 0.5 g of liver tissue (wet weight) two or three

penetrations of the liver with withdrawl of sample were necessary. The liver core samples were transferred from the cannulae into 20 mL scintillation vials containing heparinized 0.9% NaCl. Within the vials the liver samples were washed several times with heparinized 0.9% NaCl until the wash solution was clear. The samples were dried by patting with filter paper. The sample was transferred to clean 20 mL vials and dry matter determined by lyophilization.

3.5. Calculations for apparent absorption, balance, endogenous fecal Se and true absorption

Apparent absorption (AA, $\mu g\ d^{-1}$) and balance (BA, $\mu g\ d^{-1}$) of Se were computated by equations (1) and (2), respectively. Equations (3) and (4) define endogenous fecal Se (EF, $\mu g\ d^{-1}$) and true absorption (TA, $\mu g\ d^{-1}$).

$$AA = C - F \qquad (1)$$

$$BA = C - F - U \qquad (2)$$

$$EF = F \times \frac{TTMP_{feces}}{TTMP_{serum\ or\ liver}} \qquad (3)$$

$$TA = C - F + EF \qquad (4)$$

where: C = daily dietary Se consumption ($\mu g\ day^{-1}$)

F = daily fecal Se excretion ($\mu g\ day^{-1}$)

U = daily urinary Se excretion ($\mu g\ day^{-1}$).

$TTMP_{feces}$, $TTMP_{serum}$ and $TTMP_{liver}$ = tracer enrichment in feces, serum and liver, respectively

3.6. Experimental design and statistical analysis

The data for endogenous fecal Se and true absorption were subject to ANOVA by computer using the VHM version of SAS 5.03 by the general linear models procedures. All observations were interpreted by the appropriate F test on the basis of an error probability of $P < 0.05$.

The mathematical model employed was:

$$
\begin{aligned}
Y_{ijklmn} = {} & \mu + TR_i + C_j + TRxC_{ij} + A_{k(ij)} + P_l + \\
& TRxP_{il} + CxP_{jl} + TRxCxP_{ijk} + AxP_{kl(ij)} + I_m \\
& + T_n + IxT_{mn} + TRxI_{im} + CxI_{jm} \; TRxCxI_{ijm} + \\
& PxI_{lm} + TRxPxI_{ilm} + CxPxI_{jlm} + TRxCxPxI_{ijlm} + \\
& TRxT_{in} + CxT_{jn} + TRxCxT_{ijn} + PxT_{ln} + \\
& TRxPxT_{jln} + CxPxT_{jln} + TRxCxPxT_{ijln} + \\
& TRxIxT_{imn} + CxIxT_{jmn} + TRxCxIxT_{ijmn} + PxIxT_{lmn} \\
& + TRxPxIxT_{ilmn} + CxPxIxT_{jlmn} + TRxCxPxIxT_{ijlmn} \\
& + \varepsilon_{kmn((ij)l)}
\end{aligned}
$$

Where:

Y_{ijklmn} = represents the measured dependent variable

μ = the overall mean

TR_i = the trial effect where i = 1, 2

C_j = the dietary copper treatment effect where j = 1, 2

A_k = the animal effect where k = 1...11

P_l = the period effect where l = 1, 2

I_m = the route of isotope administration effect where m = 77, 82

T_n = the tissue effect where n = liver, serum

$\varepsilon_{kmn((ij)l)}$ = residual error

The other terms represent interactions between the main factors TR, C, A, P, I and T.

The same model excluding the main effects of isotope and tissue and the corresponding interactions was used for analysis of the data for apparent absorption and balance.

The analysis of variance was as follows:

Source of variation	degrees of freedom
Total	87
Trial	1
Copper	1
Trial x copper	1
Animal(trial x copper)	7
Period	1
Period x trial	1
period x copper	1
Period x trial x copper	1
Animal x period (trial x copper)	7
Isotope	1
Tissue	1
Isotope x tissue	1
Isotope x trial	1
Isotope x copper	1
Isotope x trial x copper	1
Isotope x period	1
Isotope x period x trial	1
Isotope x period x copper	1
Isotope x period x trial x copper	1
Tissue x trial	1
Tissue x copper	1
Tissue x trial x copper	1
Tissue x period	1
Tissue x period x trial	1
Tissue x period x copper	1
Tissue x period x trial x copper	1
Isotope x tissue x trial	1
Isotope x tissue x copper	1
Isotope x tissue x trial x copper	1
Isotope x tissue x period	1
Isotope x tissue x period x trial	1
Isotope x tissue x period x copper	1
Isotope x tissue x period x trial x copper	1
Residual error	42

4. RESULTS

4.1. GCMS analytical technique

The parent ion cluster of the mass spectrum of NPS contained a series of ions corresponding to the six stable isotopes of Se plus the stable isotopes of C, N and O. Ion intensities at m/z 225, 227 and 229 were determined employing the selected ion monitoring feature. The m/z values represented the most intense ions for ^{76}Se, ^{78}Se and ^{80}Se, respectively.

Data from the analyses of the stable isotope calibration sets are plotted as predicted (X, independent variable) verses observed TTMP (Y, dependent variable) in Fig. 4 and Fig. 5 for Se-76 and Se-78, respectively. For each calibration set there is a strong linear relationship and the slope and intercept do not differ from unity and zero ($P > 0.05$). This indicates the presence of a secondary Se isotope up to an enrichment level of TTMP = 83.9 does not interfere with the analysis of a sample and that there is a minimum of bias in the analysis.

The accuracy of the method for quantitative Se determination was evaluated by the analyses of two NBS standard reference materials: bovine liver (1577) and orchard leaves (1571). Good agreement between the certified and observed values was found (Table 6).

The precision of Se stable isotope enrichment was better for the measurement of Se-76 than Se-78 (Table 7). The detection limit for the measurement of isotope enrichment in a sample for

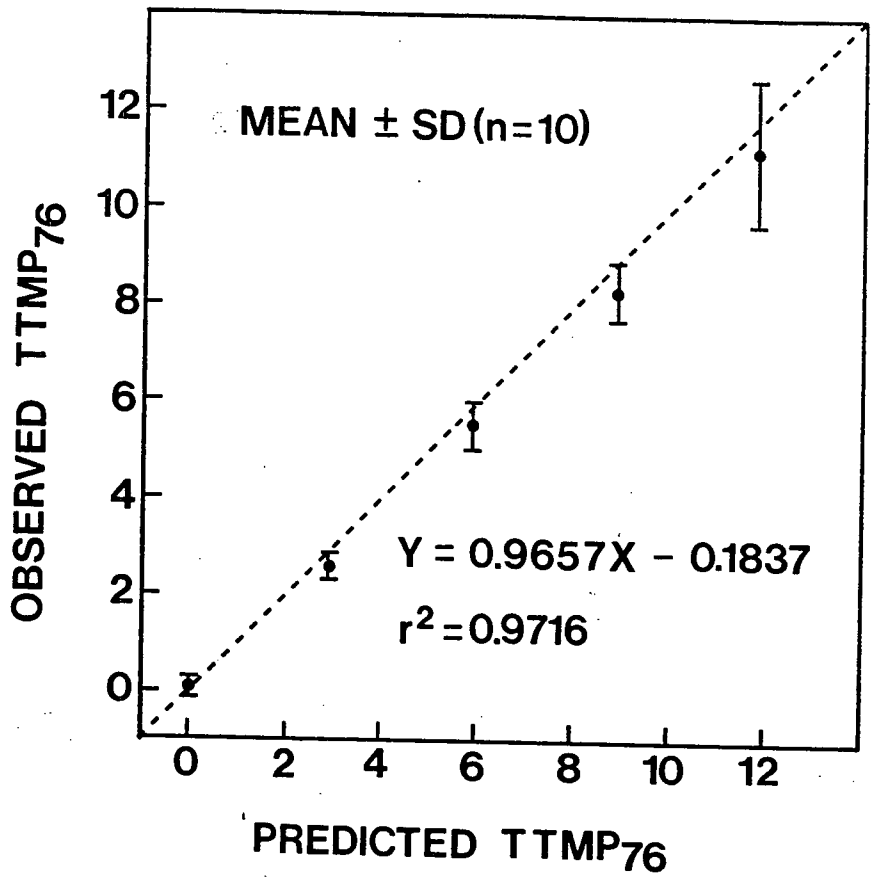


Fig. 4. Calibration curve for selenium standards enriched with Se-76 plus 5 levels of Se-78 ranging from $TTMP_{78}$ 0 to 83.9. The broken line indicates slope = 1.0 and intercept = 0.0.

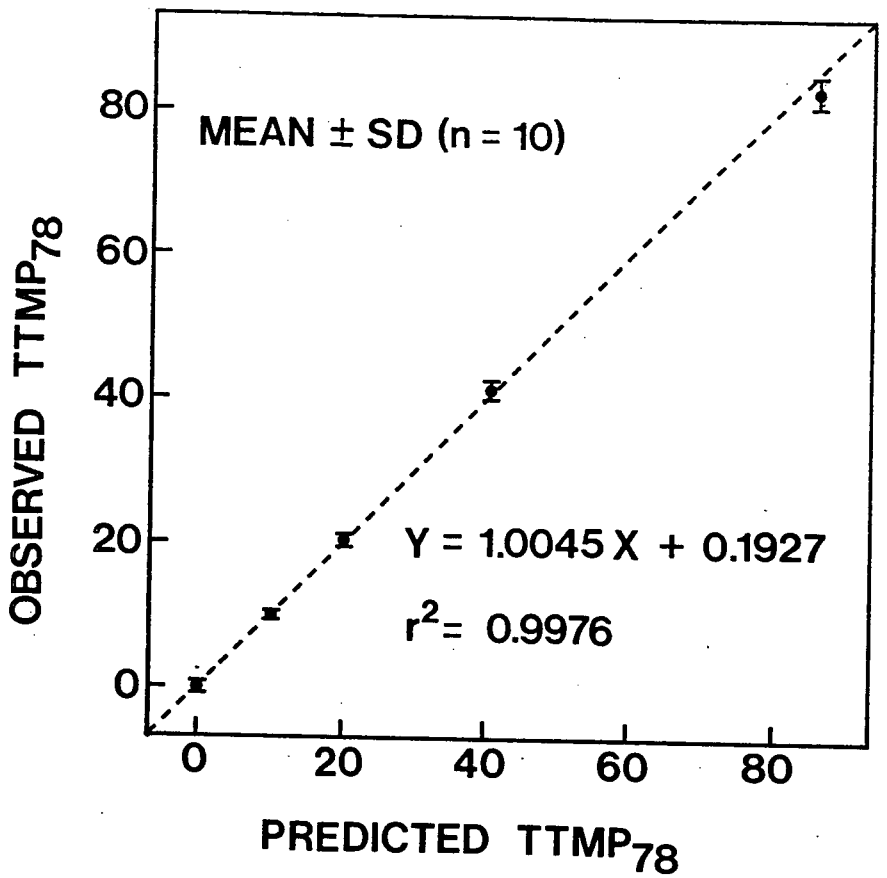


Fig. 5. Calibration curve for selenium standards enriched with Se-78 plus 5 levels of Se-76 ranging from $TTMP_{76}$ 0 to 11.8. The broken line indicates slope = 1.0 and intercept = 0.0.

Table 6. Selenium determination of standard reference materials by GCMS ($\mu g\ g^{-1}$).

Material †	Certified Se concentration ± estimate of uncertainty	Observed Se concentration ± SD (n = 3)
Bovine liver (NBS 1577)	1.1 ± 0.1	1.19 ± 0.053
Orchard leaves (NBS 1571)	0.08 ± 0.01	0.089 ± 0.004

† U. S. National Bureau of Standards (NBS), Washington DC.

Table 7. Precision of selenium stable isotope enrichment determined by GCMS. †

	$TTMP_{76}$	$TTMP_{78}$
	Selenite (sample size ~5 μg)	
Detection limit ‡	0.555	0.892
Dynamic Range §	18	11

† One sample was analyzed in triplicate on each day for 15 days.
‡ Detection limit = $t_{(0.95)(n-1)}$ x (within day variance component + between day variance component)$^{0.5}$.
§ The dilution factor that a tracer may undergo in a tissue or fluid and remain detectable [Dynamic range = (TTMP=10/detection limit)].

Se-76 and Se-78 was TTMP = 0.56 and TTMP = 0.89, respectively. Following an initial tracer enrichment of TTMP = 10, Se-76 could undergo an 18-fold dilution and remain detectable in a sample.

4.2. ICPMS analytical technique

A considerable amount of time was spent on method development for the determination of Se and multiple stable isotope enrichment of Se in biological materials by ICPMS. The results reported by Buckley et al. (1987) pertinent to decisions with regards to tracer enrichment in animals and sample preparation are included here.

The interaction between HCl concentration, Cu concentration and the intensity of the Se signal were investigated in samples containing 20 ng Se mL^{-1} of 4, 5, 6, 7, 8, 9, and 10 M HCl concentrations with Cu additions of 0, 25, 50, 100, 200 and 400 $\mu g\ mL^{-1}$. The levels of Cu were selected based on what might be expected in biological samples. The Se signal remained virtually unaffected by Cu in preparations of 9 M and 10 M HCl (Fig. 6). It was concluded for optimum sensitivity and freedom from interference by Cu, the optimum concentration of HCl for sample preparation was 9 M HCl. Preparation of samples in 9 M HCl also afforded complete reduction of Se(VI) to Se(IV). The generation of Se hydrides requires Se to be in the tetravalent state.

Usually the most abundant isotope of an element is selected to serve as the reference isotope for isotope ratio determination. However, interference from an Ar_2^+ ion at m/z 80,

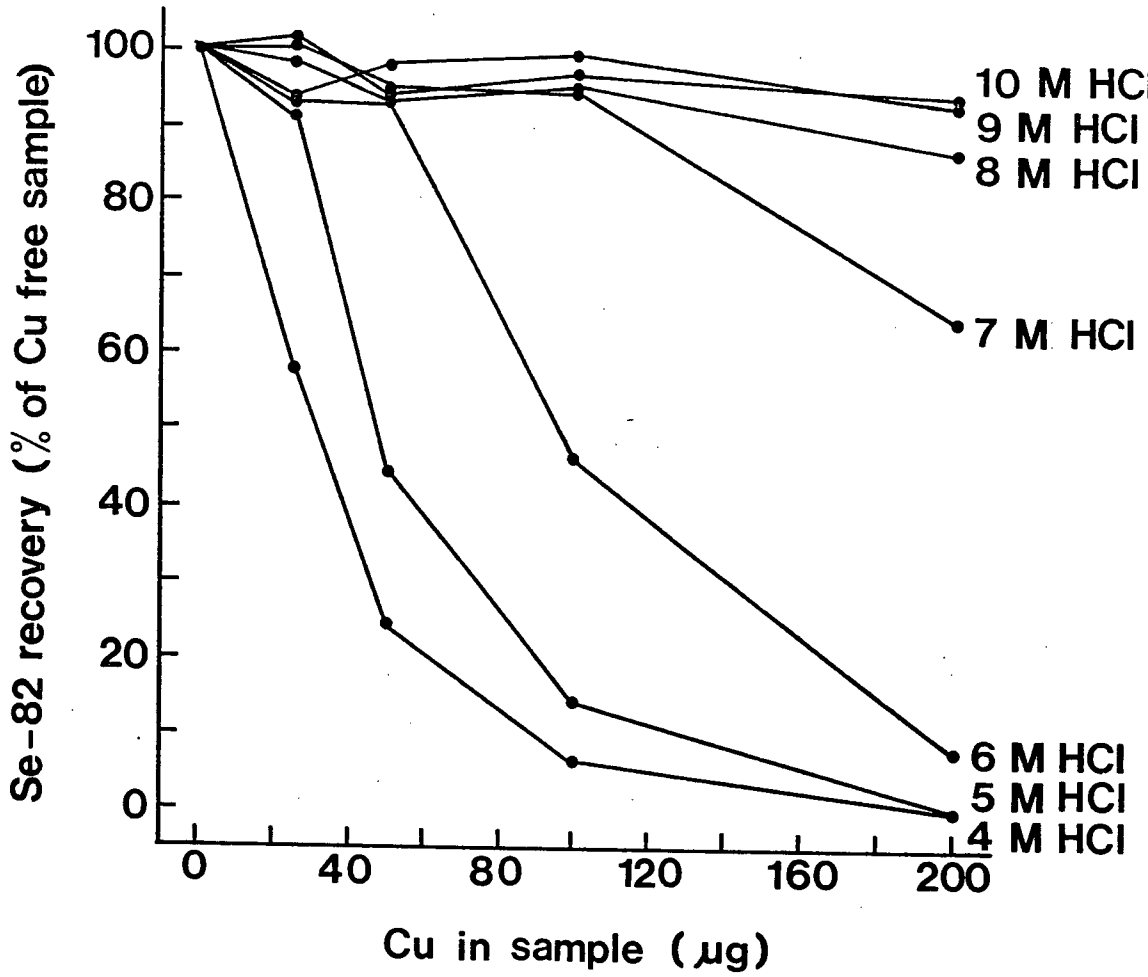


Fig. 6. Effect of HCl and Cu on the intensity of the Se signal

the most abundant isotope of Se, prevented its determination. Se-78 was therefore selected as the reference isotope. Interference in the measurement of ^{76}Se, ^{78}Se and ^{82}Se by other ions was removed by blank subtraction. A relatively high background at m/z 76 suggested Se-76 would be unsuitable as a tracer. The amount of an internal standard added to a sample for quantitative determination is several times greater than the expected level of tracer enrichment. Therefore, Se-76 was used as the internal standard.

The accuracy of selenium stable isotope enrichment was analyzed statistically by linear regression analysis of predicted verses observed isotope enrichment for Se-76, Se-77 and Se-82 The predicted TTMP (Pred TTMP) was the independent variable (X) and was fixed at graded levels of enrichment. The highest level of enrichment represented what might be expected in tracer experiments (TTMP = 10) or in samples to which an internal standard is added for quantitative analysis (TTMP = 160). The observed TTMP (Obsv TTMP) was the independent variable (Y). There was no interference of secondary Se isotope enrichment on the observed TTMP for Se-76, Se-77 and Se-82 over the ranges studied. The intercepts and slopes were pooled from the single isotope and triple isotope calibration sets for each isotope and yielded the following linear regression equations:

Se-76, $\text{Obsv TTMP}_{76} = (0.9536 \times \text{Pred TTMP}_{76}) - 0.5592$

$r^2 = 0.9998$

Se-77, $\text{Obsv TTMP}_{77} = (0.9491 \times \text{Pred TTMP}_{77}) + 0.0327$

$r^2 = 0.9998$

Se-82, $\text{Obsv TTMP}_{82} = (1.0721 \times \text{Pred TTMP}_{82}) + 0.0508$

$r^2 = 0.9996.$

Student's t-tests were employed to determine if the regression coefficients (slopes) and y-intercepts differed from unity and zero respectively. A deviation of the regression coefficient from unity was present for all isotopes. The intercept was not statistically different from zero for Se-77 and Se-82, but did deviate from zero for Se-76. To improve the accuracy for the determination of isotope enrichment and total Se, the regression equations were rearranged to solve for Pred TTMPs and used to adjust all TTMP data:

$$\text{Se-76,} \quad \text{Pred TTMP}_{76} = \frac{\text{Obsv TTMP}_{76} + 0.5592}{0.9536}$$

$$\text{Se-77,} \quad \text{Pred TTMP}_{77} = \frac{\text{Obsv TTMP}_{77}}{0.9491}$$

$$\text{Se-82,} \quad \text{Pred TTMP}_{82} = \frac{\text{Obsv TTMP}_{82}}{1.0720}$$

The accuracy of the method for quantitative Se analysis by the application of stable isotope dilution using Se-76 as the internal standard was determined by analysis of NBS standard reference materials (Table 8). There was good agreement between the observed Se concentration and the certified value for all sample materials investigated.

In sample matrices of serum, feces and urine the best precision was obtained for the measurement of $TTMP_{77}$ followed in decending order by $TTMP_{82}$ and $TTMP_{76}$ (Table 9).

Table 8. Analysis of standard reference materials for selenium by ICPMS

Material †	No. of samples	Certified Se concentration ± estimate of uncertainty	Observed Se concentration ± SD	Units
Bovine liver (NBS 1577)	3	1.1 ± 0.1	1.1 ± 0.0004	$\mu g\ g^{-1}$
Bovine serum (NBS 8419)	6	16 ± 2	15 ± 0.3	$ng\ mL^{-1}$
Freeze dried urine (NBS 2670)	3	30 ± 8	30 ± 2.2	$ng\ mL^{-1}$
Rice flour (NBS 1568)	6	0.4 ± 0.1	0.32 ± 0.007	$\mu g\ g^{-1}$

† U.S. National Bureau of Standards (NBS), Washington DC.

Table 9. Precision of selenium stable isotope enrichment determined by ICPMS †

	$TTMP_{76}$	$TTMP_{77}$	$TTMP_{82}$
	Serum (sample size = 430 ng)		
Detection limit ‡	0.145	0.050	0.078
Dynamic range §	69	200	128
	Feces (sample size = 244 ng)		
Detection limit ‡	0.355	0.039	0.070
Dynamic range §	28	256	142
	Urine (sample size = 438 ng)		
Detection limit ‡	0.699	0.017	0.069
Dynamic range §	14	588	145

† Three samples were prepared and analyzed each day for 4 (urine) or 7 days (serum and feces). Se concentrations in serum, feces and urine were 86 ng mL^{-1}, 244 ng g^{-1} and 73 ng mL^{-1}, respectively.

‡ Detection limit = $t_{(0.95)(n-1)}$ x (within day variance component + between day variance component)$^{0.5}$.

§ The dilution factor that a tracer may undergo in a tissue or fluid and remain detectable [Dynamic range = (TTMP=10/detection limit)].

4.3. Trial I

4.3.1. Animals and diet

Cow 8219 and 7939 weighed 642 and 711 kg respectively. Cow 7939 remained standing during much of the first balance period. During the second period the cow appeared weak and developed a slight elevation of temperature. The cow was moved into a box stall where it continued to receive the experimental diet. It recovered over the next two days, and so remained on trial for the collection of tissues and fluids. Balance data for this animal was obtained for period 1 only.

The amounts of most components in the orchardgrass hay met the recommended levels for dry pregnant cows (NAS-NRC 1978) (Table 10). The exceptions were Se, Cu, and Zn whose dietary concentrations fell below the current recommended dietary levels of 0.1 mg kg^{-1}, 10 mg kg^{-1} and 40 mg kg^{-1}, respectively. The Se concentration of the orchardgrass hay was 0.035 $\pm$ 0.002 mg kg^{-1}. The concentration of Se in the water was 1.03 μg L^{-1}.

4.3.2. Selenium content of tissues and fluids

At the time of slaughter, cow 8219 was found to be carrying a fetus of ~5 months of age. The whole fetus and several tissues were collected, subsampled and analyzed as described for tissues of the mature animals.

Despite the different physiological states of the two non-lactating cows (pregnant and non-pregnant) the Se

Table 10. Composition of orchardgrass hay (dry matter basis) (Trial 1)

Component	
Dry matter (%) †	89.3 ± 0.7
Crude protein (%) †	12.3 ± 0.96
Acid detergent fiber (%) †	34.8 ± 0.33
Calcium (%)	0.36
Phosphorus (%)	0.24
Magnesium (%)	0.16
Sulfur (%)	-
Iron (mg kg^{-1})	116
Copper (mg kg^{-1}) †	6.2 ± 0.5
Manganese (mg kg^{-1})	90
Zinc (mg kg^{-1})	16
Molybdenum (mg kg^{-1}) †	0.63 ± 0.007
Selenium (mg kg^{-1}) †	0.035 ± 0.002

† Mean ± SD, n=3.

concentrations of the tissues and fluids were similar (Table 11).

Based on the mean for the two animals (Table 12), the highest concentration of Se was found in the kidney cortex (8.07 $\mu g\ g^{-1}$). Intermediate levels (0.87 - 1.82 $\mu g\ g^{-1}$) were found in the kidney medulla, spleen, pancreas, small intestine, heart, lung and liver. The Se concentration of the cardiac muscle (1.0 $\mu g\ g^{-1}$) was greater than that of the smooth muscle of the gastrointestinal tract and uterus (0.40 - 0.65 $\mu g\ g^{-1}$) and the skeletal muscle (0.44 $\mu g\ g^{-1}$). The lowest Se concentrations were found in the bile (0.06 $\mu g\ g^{-1}$), bone and adipose tissue (0.02 $\mu g\ g^{-1}$). The serum contained 0.048 $\mu g\ mL^{-1}$.

Of the fetal tissues analyzed (Table 13), the highest Se concentration was found in the liver (1.7 $\mu g\ g^{-1}$). The Se concentration in the fetal liver and hide was greater than twice that of the corresponding tissues in the dam (8219). In contrast, values for the fetal kidney and muscle were lower than the corresponding tissues in dam.

The quantities of Se associated with the various tissues (Table 12) were estimated from the mean tissue Se concentrations and from data of anatomical structures for Holstein cows (Matthews et al. 1975) and estimates of carcass composition (R. J. Forrest, personal communication: subdivision of total carcass weight into: muscle, 55%; adipose tissue, 25%; and bone, 20%) The weight of the uterus was estimated from the weight of the fetus (5.1 kg) and placenta (1.6 kg) and the relative weights of the conceptus and associated tissues in sheep (Langlands et al.

1982; Table 2, 5th stage). The estimate of total Se for hair and hide was calculated from the Se concentration of the hide (0.22 $\mu g\ g^{-1}$) found in the present study. Perry et al. (1977) reported Hereford steers fed a diet containing 0.08 mg Se kg^{-1} had hair Se concentrations of 0.30 $\mu g\ g^{-1}$. Puls (1981) reported the Se concentration of hair ranged from 0.06 to 0.23 $\mu g\ g^{-1}$ for cattle on diets containing <0.10 mg Se kg^{-1}. The animals in the present study were consuming a diet containing 0.035 mg Se kg^{-1}, therefore, 0.22 μg Se g^{-1} was assumed to provide a representative estimate of the Se concentration in the hair. The Se concentration in blood was estimated from the Se concentration in serum and from Scholz and Hutchinson (1979) whom reported 72.8% of whole blood Se in dairy cows was in the cellular component.

Summation of the estimated quantities of Se associated with the various tissues and fluids, yielded a total body Se content of 44.4 mg for the non-lactating dairy cows consuming a low Se diet (0.035 $\pm$ 0.002 mg kg^{-1}). Of the total body Se, about 47%, 17%, 6%, 5% and 3% was associated with skeletal muscle, hide and hair, digestive tract, liver, and kidney, respectively. The blood accounted for 6% of the total body Se. The Se content of the 5-month old fetus was 0.3 mg and made up 0.6% of the body Se of the mature cow (Table 13). The fetal liver and kidney contained 11% and 3%, respectively, of the fetal body Se.

4.3.3. Distribution of Se-76 in tissues and fluids

Se-76 enrichment in tissues and fluids was variable as

indicated in Fig. 7. The enrichment of Se-76 in the adipose tissue and bone was below the detection limit for GCMS analysis (detection limit, TTMP = 0.56). The tissues and fluids considered influential and/or contributors to endogenous fecal Se are: serum, the epithelium of the stomach (including the rumen, reticulum, omasum and abomasum), liver, bile, pancreas, small intestine and colon. There existed little variability of tracer enrichment amongst these tissues and fluids with TTMPs of 9.9 - 12.4 and 8.7 - 13.3 for cow 8219 and 7939, respectively.

The uterus (Fig. 7), placenta and tissues of the fetus (Fig. 8) from cow 8219 show a high level of tracer enrichment with TTMPs ranging from 11.2 to 13.4. The tracer enrichment in the skeletal muscle of the fetus (TTMP = 12.2) was > 6 times the level present in the dam (TTMP = 1.8). The hide of the fetus (TTMP = 11.9) was also enriched with Se-76 to a greater degree than the hide of the dam (TTMP = 8.6).

Table 11. Selenium concentration in tissues and fluids of cow 8219 and cow 7939 (dry matter basis)

Tissue	No. of sites	Cow 8219 Se ($\mu g\ g^{-1}$)	Cow 8219 SD†	Cow 7939 Se ($\mu g\ g^{-1}$)	Cow 7939 SD†
kidney cortex	1	8.949		7.191	
kidney medulla	1	1.868		1.775	
spleen	1	1.491		1.219	
pancreas	1	0.890		1.369	
lung	1	0.952		0.952	
liver	5	0.773	0.026	0.966	0.042
udder	1	0.705		0.625	
uterus epithelium	1	0.713			
uterus smooth muscle	1	0.401			
stomach epithelium	4	0.606	0.022	0.716	0.078
stomach smooth muscle	4	0.586	0.182	0.709	0.071
small intestine	1	1.155		1.090	
colon	1	0.512		0.948	
skeletal muscle	6	0.443	0.025	0.433	0.033
adipose	2	0.014	0.004	0.022	0.007
bone	3	0.021	0.002	0.025	0.001
bile	1	0.045		0.077	
serum‡	1	0.041		0.054	

† SD for sample sites.
‡ $\mu g\ mL^{-1}$

Table 12. Estimated selenium content in tissues, fluids and the whole body of non-lactating dairy cows

Tissue	Mean Se concentration ($\mu g\ g^{-1}$ DM)	Estimated total Se (mg)	Percent of total body Se
Kidney	4.95	1.5	3.3
cortex	8.07		
medulla	1.82		
Spleen	1.36	0.3	0.7
Pancreas	1.13	0.2	0.4
Heart	1.0	0.6	1.3
Lung	0.95	0.9	2.1
Liver	0.87	2.1	4.8
Udder	0.67	1.5	3.4
Uterus	0.56	0.3	0.7
Digestive tract	0.75	2.8	6.2
Skeletal muscle	0.44	20.8	46.8
Adipose	0.02	1.2	2.8
Bone	0.02	1.8	4.1
Hide and hair	0.22 †	7.4	16.7
Serum	0.048	0.7	1.7
Blood	2.75	2.7	6.2

† Mean Se concentration for hair.

Table 13. Selenium content in the placenta and tissues of the 5-month old fetus from cow 8219

Tissue	Se ($\mu g\ g^{-1}$ DM)	Estimated total Se (mg)	Percent of dam's total body Se
Placenta	0.52	0.08	0.2
Kidney (whole)	1.47		
Liver	1.77		
Skeletal muscle †	0.28 ± 0.037		
Hide	0.45		
Fetus (whole)	0.30 ‡	0.3	0.6

† Mean ± SD of longissimus dorsi, biceps femoris and superficial pectoral.
‡ Se concentration of whole fetus less kidneys, liver, and sections of muscle and hide.

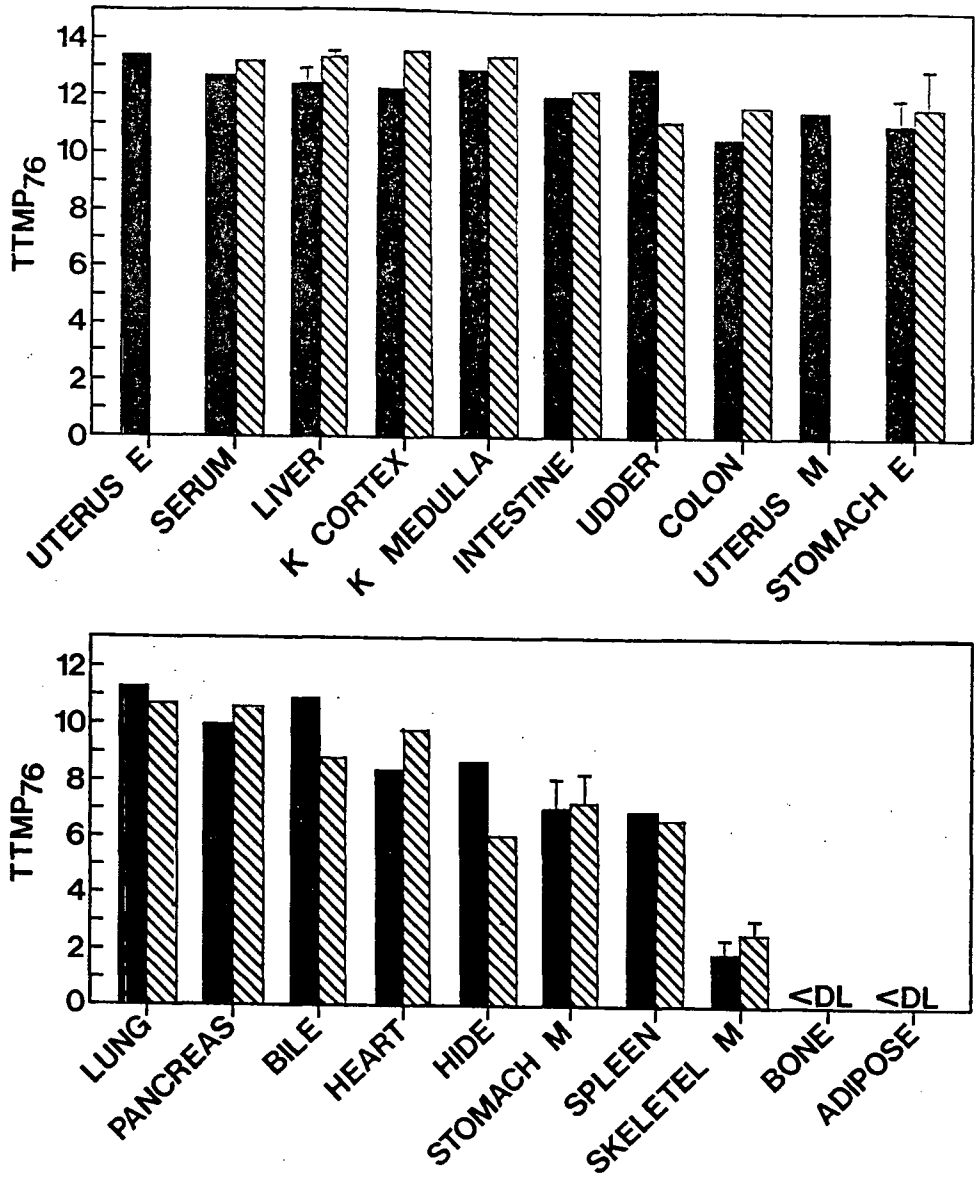


Fig. 7. Se-76 enrichment (TTMP) in tissues and fluids of cow 8219 (▓▓) and cow 7939 (▨). The SD are for the sample sites. (E = epithelium; M = muscle; K = kidney; DL = detection limit, TTMP = 0.56)

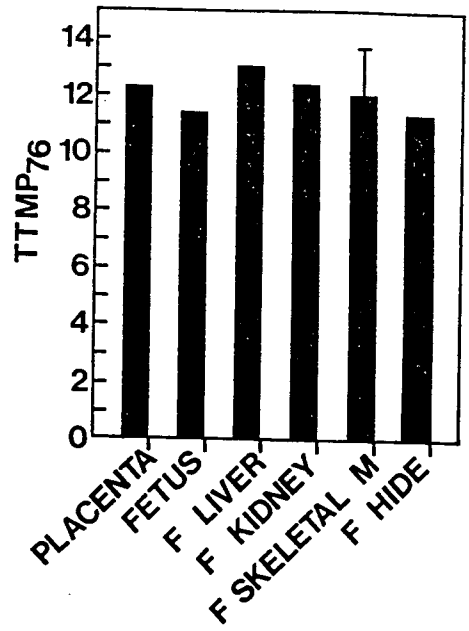


Fig. 8. Se-76 enrichment (TTMP) in the placenta and tissues of the 5-month old fetus from cow 8219.

4.3.4. Absorption and endogenous fecal excretion of selenium

Incomplete collection of urine resulted in urine contamination of the 24-h fecal collections for cow 8219 on day 1 of Period 1 and day 4 of Period 2, and for cow 7939 on day 4 of Period 1. Therefore, the number of samples composited for feces and urine for each balance period was reduced from five to four.

The average daily feed intake, and fecal and urine excretion for each period are given in Table 14. The average dry matter percent of the feces was 17.3%. Mechanical failure of the regulator valves of the water bowls prevented the measurement of water intakes.

The average daily Se intake and fecal Se excretion for the two cows are given in Table 15. Selenium was unable to be determined in the urine by GCMS analysis. The endogenous fecal excretion of Se by cow 8219 was 109 - 111 $\mu g\ d^{-1}$ and contributed 22 - 23% of the total Se excreted in the feces (Table 15). For the non-pregnant cow (7939) with a lower Se intake, the daily endogenous fecal excretion of Se was 183 $\mu g\ d^{-1}$, amounting to 36% of the total fecal Se (Table 15).

Apparent absorption of Se for the two cows was negative (Table 16). True absorption expressed as a percentage of the Se intake by cow 8219 was 15 - 16% (Table 16). There was very good agreement between the measurements taken in each period for this animal. The true absorption of Se by cow 7939 was lower and equal to 10% of the total Se intake (Table 16).

Table 14. Feed intake and feces and urine excretion in Period 1 and 2 † (Trial I)

Cow No.	Period ‡	Feed intake § ($kg\ d^{-1}$)	Feces output § ($kg\ d^{-1}$)	Urine output ($L\ d^{-1}$)
8219	1	12.49 ± 1.43	4.58 ± 0.07	15.14 ± 0.83
8219	2	13.13 ± 1.36	4.68 ± 0.50	12.27 ± 1.92
7939	1	10.04 ± 2.07	3.80 ± 0.17	17.45 ± 1.84

† Mean ± SD, n=4.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.
§ Dry matter basis.

Table 15. Intake and feces excretion of selenium ($\mu g\ d^{-1}$) (Trial I)

Cow No.	Period ‡	Se Intake † (C)	Feces Se (F)	Endogenous fecal Se (EF)	Endogenous fecal Se (% of intake)
8219	1	448 ± 51	484	109	23%
8219	2	471 ± 49	509	111	22%
7939	1	360 ± 74	507	183	36%

† Mean ± SD, n=4.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.

Table 16. Apparent and true absorption of selenium ($\mu g\ d^{-1}$) (Trial I)

Cow No.	Period ‡	Se Intake † (C)	Apparent Se Absorption (AA)	True Se Absorption (TA)	True Se Absorption (% of intake)
8219	1	448 ± 51	-36	73	16%
8219	2	471 ± 49	-38	73	15%
7939	1	360 ± 74	-147	36	10%

† Mean ± SD, n=4.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.

4.4. Trial II and III

In Trial II one cow (8337) was diagnosed by a veterinarian with pneumonia. This animal was removed and the trial continued with 5 animals.

4.4.1. Experimental diets

The basal diet provided the recommended dietary allowance for all nutrients analyzed (Table 17), with the exception of zinc (37 mg kg^{-1}) which was below the recommended concentration of 40 mg kg^{-1} (NAS-NRC 1978). The Se concentration of the diet was 0.188 ± 0.006 mg kg^{-1}. The level of Cu supplementation achieved (Table 18) was 15 - 17 mg kg^{-1}. The total Cu concentration of Treatment 2 was 29 - 30 mg kg^{-1}. The Cu content of Treatment 1 (control) was expected to be below 10 mg kg^{-1}, but contained 13 - 14 mg Cu kg^{-1}. The concentration of Se in the water was 1.24 ± 0.21 μg L^{-1}.

4.4.2. Feed and water intake and urine and feces excretion

The mean body weight of the cows (Table 19) of Treatment 2 (681 ± 31) was greater than that of Treatment 1 (651 ± 32). The larger body weight of the cows on Treatment 2 is reflected in greater daily feed and water intakes and fecal and urine outputs (Table 19).

Table 17. Compositon of basal diet used in Trial II and III (dry matter basis) †

Component	
Dry matter (%)	91.1 ± 0.4
Crude protein (%)	16.8 ± 0.1
Acid detergent fiber (%)	33.3 ± 0.5
Calcium (%)	0.48 ± 0.02
Phosphorus (%)	0.31 ± 0.01
Magnesium (%)	0.26 ± 0.01
Potassium (%)	1.66 ± 0.9
Sulfur (%)	0.36 ± 0.005
Iron (mg kg^{-1})	586 ± 70
Manganese (mg kg^{-1})	109 ± 2
Zinc (mg kg^{-1})	37 ± 1.3
Molybdenum (mg kg^{-1})	0.42 ± 0.001
Selenium (mg kg^{-1})	0.188 ± 0.006

† Mean ± Pooled SD for Treatment 1 and 2 of Trial II and III, n=4.

Table 18. Copper concentration of the experimental diets (dry matter basis) † (Trial II and III)

	Copper (mg kg^{-1})
Trial II	
Treatment 1 (control)	14 ± 1.8
Treatment 2	29 ± 1.9
Trial III	
Treatment 1 (control)	13 ± 0.4
Treatment 2	30 ± 2.0

† Mean ± SD, n=3.

Table 19. Body weight, feed and water intake, and feces and urine excretion for cows on Treatment 1 and 2 †
(Trail II and III)

Treatment	n	Body Weight (kg)	Feed Intake ‡ (kg d^{-1})	Water Intake (L d^{-1})	Feces Output ‡ (kg d^{-1})	Urine Output (L d^{-1})
1	5	651 ±32	12.54 ±0.88	62.19 ±3.94	5.13 ±0.41	12.70 ±0.83
2	6	681 ±31	14.94 ±0.42	72.19 ±2.98	6.01 ±0.20	14.32 ±0.40

† Mean ± SEM.
‡ Dry matter basis.

4.4.3. Selenium concentration in serum and liver

The concentration of Se ($\mu g\ mL^{-1}$) in the serum of the animals of Treatment 1 (0.051 ± 0.002) was higher than that of animals of Treatment 2 (0.046 ± 0.001) one day prior to the trial ($P < 0.05$). The Se concentration during Period 1 and 2 were also higher for those animals on Treatment 1 (Table 20). No difference ($P > 0.05$) was found between periods for Treatment 1 or Treatment 2. The mean serum Se concentration for the periods for both Treatment 1 (0.059 ± 0.001) and Treatment 2 (0.054 ± 0.001) were higher than the corresponding concentrations prior to the trial ($P < 0.05$).

The Se concentration in the liver for animals on Treatment 1 and Treatment 2 were 1.0 + 0.05 and 0.97 + 0.03 $\mu g\ g^{-1}$, respectively, and were not significantly different ($P > 0.05$).

4.4.4. Isotope enrichment in tissues and excrement

There was greater enrichment of Se-82 in all the materials (serum, liver, feces and urine) in accordance with dosage and route of administration (Table 21). The values for the liver are for the enrichment of Se-77 and Se-82 occurring 2 days following the completion of Period 2. Cu did not influence the level of enrichment of either isotope ($P > 0.05$). There was an effect of period ($P < 0.05$) resulting in lower isotope enrichment in serum, feces and urine in Period 2.

Table 20. Selenium concentration ($\mu g\ mL^{-1}$) 1 day prior to the trial and during Period 1 and 2 † (Trial II and III).

	1 day prior to trial	Period 1 ‡	Period 2 ‡	Mean of Period 1+2
Treatment 1	0.051 ±0.002a	0.058 ±0.002c	0.059 ±0.002c	0.059 ±0.001
Treatment 2	0.046 ±0.001b	0.054 ±0.001ad	0.054 ±0.002ad	0.054 ±0.001

† Mean ± SEM, n=5 for Treatment 1 and n=6 for Treatment 2.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.
a,b,c,d Means with the same letter are not significantly different (P>0.05).

Table 21. Se-77 and Se-82 enrichment (TTMP) in serum, liver feces and urine † (Trial II and III)

Tissue	Treatment	Se-77		Se-82	
		Period 1 ‡	Period 2 ‡	Period 1 ‡	Period 2 ‡
Serum	1	1.56 ±0.12	1.27 ±0.10	2.34 ±0.09	1.99 ±0.08
	2	1.66 ±0.07	1.33 ±0.05	2.38 ±0.05	1.96 ±0.05
Liver §	1		1.38 ±0.15		2.36 ±0.13
	2		1.47 ±0.06		2.45 ±0.05
Feces	1	0.17 ±0.013	0.15 ±0.019	0.23 ±0.02	0.20 ±0.03
	2	0.17 ±0.007	0.12 ±0.008	0.23 ±0.02	0.18 ±0.004
Urine	1	1.08 ±0.12	0.88 ±0.11	1.59 ±0.10	1.37 ±0.13
	2	1.03 ±0.04	0.85 ±0.03	1.53 ±0.03	1.27 ±0.02

† Mean ± SEM, n=5 for Treatment 1 and n=6 for Treatment 2.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.
§ Isotope enrichment 2 days following the completion of Period 2.
Means for Se-77 and Se-82 enrichment in serum, feces and urine for periods are significantly different ($P<0.05$).

4.4.5. Apparent absorption and balance of selenium

The Se intake from the water amounted to ~3.2% of the overall mean Se intake from the feed and was not included in absorption or balance calculations.

The measurements of apparent absorption were variable as indicated in Table 22. The mean apparent absorption (% of intake) for the animals receiving the supplemental Cu (5.1 ± 1.5%) was greater than for those receiving the control diet (0.9 ± 11.5%) but was not significantly different ($P > 0.05$). There was no effect of period ($P > 0.05$) although the mean for Period 1 (5.4 ± 1.8%) was greater than for Period 2 (1.1 ± 1.2%) The overall mean for apparent absorption of Se (% of intake) was 3.2 ± 1.2%.

There was no effect of Cu or period on Se balance ($P > 0.05$) (Table 22). The cows on this experiment were in a negative balance with an overall mean loss of 175 ± 31 μg Se d^{-1} from the body Se stores.

There was little variation between treatments or periods for the daily urinary Se excretion (Table 22) which accounted for an overall mean loss of 267 ± 10 μg d^{-1}. Of the total Se excreted, 10.6 % was excreted in the urine.

Table 22. Apparent absorption and balance of selenium ($\mu g\ d^{-1}$) † (Trial II and III)

	Treatment 1		Treatment 2		Overall Mean
	Period 1 ‡	Period 2 ‡	Period 1 ‡	Period 2 ‡	
Se Intake (C)	2398 ±239	2328 ±211	2859 ±107	2736 ±104	2600 ±91
Fecal Se (F)	2350 ±225	2317 ±191	2624 ±129	2683 ±122	2508 ±84
Apparent Se Absorption(AA)	48 ±72	11 ±35	234 ±50	53 ±49	92 ±31
Apparent Se Absorption §	1.8 ±2.8	0.05 ±1.6	8.3 ±1.7	2.0 ±1.8	3.2 ±1.2
Urinary Se (U)	269 ±31	258 ±34	269 ±11	268 ±8	267 ±10
Se Balance(BA)	-221 ±58	-247 ±43	-35 ±60	-215 ±53	-175 ±31

† Mean ± SEM, n=5 for Treatment 1 and n=6 for Treatment 2.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.
§ % of intake.
Means for treatments and for periods were not significantly different ($P>0.05$).

4.4.6. Endogenous fecal excretion and true absorption of Se

The endogenous fecal Se was determined from the analysis of serum and liver enriched with Se-77 (oral route of administration) and Se-82 (intravenous route of administration) yielding 4 estimates. These estimates of endogenous fecal Se were then used to calculate 4 estimates of true Se absorption (TA = C - F + EF).

The period effect was not significant ($P > 0.05$) for either route of administration when endogenous excretion (Table 23) and true absorption (Table 24) were estimated from enrichment of the tracers in serum. It was significant when estimated from the enrichment of the tracers in the liver. The estimate for $TTMP_{liver}$ for Period 1 was higher than for Period 2 for both routes of administration ($P < 0.05$). The liver sample was taken 2 days following the completion of the second period and therefore the tracer enrichment did not accurately reflect that which would have been present in Period 1. As natural Se continues to enter the system the quantity of the tracer in tissues becomes diluted. Thus, the tracer enrichment in the liver at the end of the two periods would be lower than that which would have been present during the first period. The result is a larger ratio of $TTMP_{feces}/TTMP_{liver}$ and an overestimation of the endogenous fecal Se excreted in Period 1. For this reason, the effect of route of isotope administration and the tissue index on the estimate of endogenous fecal Se (Table 25) and true absorption (Table 26) were determined from

the observations of Period 2. The ANOVA showed no effect of Cu on the endogenous fecal Se excreted and on true absorption. There was no difference between the estimates of endogenous fecal Se ($\mu g\ d^{-1}$) when determined from tracer enrichment in the serum (256 ± 13) or liver (235 ± 14) following oral administration of the tracer (Se-77) and when determined from enrichment in serum (241 ± 15) following intravenous administration of the tracer (Se-82). The mean of these three measures for endogenous fecal Se excretion was 244 ± 6 $\mu g\ d^{-1}$. This represented 9.7% of the total daily fecal Se excreted. The estimate of endogenous fecal Se determined from the enrichment in the liver (197 ± 11) following intravenous administration of the tracer was significantly less ($P < 0.05$) than the estimates obtained from the other three methods of measurement.

There were no significant differences ($P > 0.05$) among the four estimates of true Se absorption for each treatment (Table 26). True absorption determined from the enrichment in liver following intravenous administration, did however, tend to be lower than the means determined from enrichment in serum or liver following oral administration and from enrichment in serum following intravenous administration of the tracer. The overall mean for true absorption (% of intake) of Se in the dairy cattle of Trial II and III was 11 ± 1.5%.

Table 23. Endogenous fecal selenium ($\mu g\ d^{-1}$) †
(Trial II and III)

Route of Administration	Tissue	Treatment 1		Treatment 2	
		Period 1 ‡	Period 2 ‡	Period 1 ‡	Period 2 ‡
Oral (Se-77)	Serum	256 ±32a	258 ±13a	267 ±23a	254 ±23a
	Liver	294 ±41a	242 ±20b	300 ±26a	229 ±21b
Intravenous (Se-82)	Serum	239 ±34a	231 ±29a	255 ±29a	248 ±17a
	Liver	238 ±34a	194 ±21b	248 ±27a	199 ±14b

† Mean ± SEM, n=5 for Treatment 1 and n=6 for Treatment 2.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.
a,b Means followed by the same letter within a row are not significantly different (P>0.05).

Table 24. True absorption of selenium ($\mu g\ d^{-1}$) (Values in parentheses are means for true absorption of selenium expressed as % of selenium intake) † (Trial II and III)

Route of Administration	Tissue	Treatment 1		Treatment 2	
		Period 1 ‡	Period 2 ‡	Period 1 ‡	Period 2 ‡
Oral (Se-77)	Serum	304 ±73a (12.4 ±2.2)	269 ±41a (11.4 ±1.1)	501 ±51a (17.6 ±1.8)	307 ±54a (11.2 ±2.0)
	Liver	342 ±78a (13.9 ±2.1)	253 ±42b (10.6 ±1.2)	534 ±51a (18.8 ±1.8)	281 ±50b (10.3 ±1.8)
Intravenous (Se-82)	Serum	287 ±67a (11.7 ±2.1)	242 ±56a (10.2 ±1.9)	490 ±31a (17.2 ±1.2)	251 ±43a (9.2 ±1.6)
	Liver	286 ±70a (11.6 ±2.1)	205 ±49b (8.6 ±1.7)	482 ±30a (17.0 ±1.2)	301 ±42b (11.0 ±1.6)

† Means ± SEM, n=5 for Treatment 1 and n=6 for Treatment 2.
‡ Period 1 = days 15-20 and Period 2 = days 20-25.
a,b Means followed by the same letter within a row are not significantly different (P>0.05).

Table 25. Effect of route of isotope administration and tissue analyzed on the calculation of endogenous fecal selenium ($\mu g\ d^{-1}$) (Values in parentheses are means for endogenous fecal selenium expressed as % of fecal selenium) † (Trial II and III)

Route of Administration	Tissue	Treatment 1	Treatment 2	Overall Mean
Oral (Se-77)	Serum	258 ± 13a (11.4 ± 0.8)	254 ± 23a (9.5 ± 0.8)	256 ± 13 (10.3 ± 0.6)
	Liver	242 ± 20a (10.6 ± 0.8)	229 ± 21a (8.5 ± 0.6)	235 ± 14 (9.4 ± 0.6)
Intravenous (Se-82)	Serum	231 ± 29a (10.2 ± 1.2)	248 ± 17a (9.2 ± 0.4)	241 ± 15 (9.7 ± 0.6)
	Liver	194 ± 21b (8.5 ± 0.9)	199 ± 14b (7.4 ± 0.2)	197 ± 11 (7.9 ± 0.4)

† Mean ± SEM for Period 2 (days 20-25), n=5 for Treatment 1 and n=6 for Treatment 2.
a,b Means followed by the same letter are not significantly different (P>0.05).

Table 26. Effect of route of isotope administration and tissue analyzed on the calculation of true absorption of selenium ($\mu g\ d^{-1}$) (Values in parentheses are means for true absorption of selenium expressed as % of selenium intake) † (Trial II and III)

Route of Administration	Tissue	Treatment 1	Treatment 2	Overall Mean
Oral (Se-77)	Serum	269 ± 41	307 ± 54	290 ± 33
		(11.4 ± 1.1)	(11.2 ± 2.0)	(11.3 ± 1.2)
	Liver	253 ± 42	281 ± 50	268 ± 32
		(10.6 ± 1.2)	(10.3 ± 1.8)	(10.4 ± 1.1)
Intravenous (Se-82)	Serum	242 ± 56	301 ± 42	274 ± 34
		(10.2 ± 1.9)	(11.0 ± 1.6)	(10.7 ± 1.2)
	Liver	205 ± 49	252 ± 43	230 ± 31
		(8.6 ± 1.7)	(9.2 ± 1.6)	(8.9 ± 1.1)

† Mean ± SEM for Period 2 (days 20-25), n=5 for Treatment 1 and n=6 for Treatment 2.
Means for true absorption of selenium are not significantly different ($P>0.05$).

5. DISCUSSION

5.1. Selenium stable isotopes as tracers

A minor systematic bias was present in the analysis of Se stable isotopes by ICPMS. This was reflected by the deviation of the regression coefficient from unity for all isotopes, and the deviation of the intercept from zero for Se-76. The systematic bias observed in the calibration lines was not due to cross-contamination or memory effects from previously run samples (Buckley et al. 1987). To improve the accuracy of Se stable isotope measurements by ICPMS the bias was removed in the analysis of biological samples and NBS reference materials by applying the appropriate correction factors based on the regression equations:

$$\text{Se-76,} \quad \text{Pred TTMP}_{76} = \frac{\text{Obsv TTMP}_{76} + 0.5592}{0.9536}$$

$$\text{Se-77,} \quad \text{Pred TTMP}_{77} = \frac{\text{Obsv TTMP}_{77}}{0.9491}$$

$$\text{Se-82,} \quad \text{Pred TTMP}_{82} = \frac{\text{Obsv TTMP}_{82}}{1.0720}$$

The technique of hydride generation is subject to interferences between the hydride forming elements themselves and from other matrix elements. The production of gaseous hydrides of Se are chemically inhibited by the presence of commonly

occurring metals such as Cu. Vijan and Leung (1980) reported that in samples of high HCl concentration, chlorocomplexes were formed with the metals (Cu, Ni and heavy metals) resulting in the virtual elimination of interference. They reported an acid concentration of 7.5 M HCl was necessary to achieve optimum sensitivity and freedom from interference by heavy metals. In the present study 9 M HCl was necessary to eliminate the interference by Cu on the Se signal intensity (Fig. 6). The need for a higher acid concentration for the elimination of interference found in the present study may be due to the higher concentration of Cu in the samples. The samples contained up to 8 mg Cu mL^{-1} whereas the samples of Vijan and Leung (1980) contained up to 30 mg Cu mL^{-1}. The preparation of samples with 9 M HCl also offered the advantage of complete reduction of Se(VI) to Se(IV), the chemical form of Se required for the production of Se hydrides. This eliminates the need for further sample processing, such as heating, for the conversion of Se to the appropriate valency state.

The elemental compositions of the sample matrices of interest differed sufficiently such that interference problems associated with each matrix were unique. The interference resulted in minor deviations of the Se isotopic ratios from the published values for natural isotopic ratios. It is therefore advisable to measure the isotopic ratios of natural Se in a particular matrix and use the measured values, rather than the published values, to calculate tracer enrichment in unknown

samples of the same matrix.

Analytical chemistry of Se in matrices of interest is relatively complex because of a number of issues which include: the multivalent state of Se and the relative ease of conversion among oxidation states; the likely existence of multiple forms of Se and their relative liability and volatility; and the need for conversion of all Se moieties into a similar chemical species (Se(IV) in this case) (Janghorbani et al. 1985). The methods described herein for the quantitative analysis of Se were found to be accurate as indicated by good agreement between the certified values of NBS reference materials and the observed values (Table 6 and Table 8). Janghorbani et al. (1982b) reported some chemical forms of Se (e.g. trimethylselenonium) in urine were resistant to digestion and conversion to Se(IV). The method for the preparation and analysis of samples by ICPMS proved capable of overcoming any such resistance as demonstrated by the results of the analyses of NBS freeze dried urine (Table 8). In contrast, Se was not detected in urine samples analyzed by GCMS. The digestion aid used in the preparation of the samples for GCMS analysis was prepared with hydrated magnesium nitrate. The presence of water in the digestion aid may have decreased the strength of the aid for oxidation of Se in the urine sample. It could have also been possible that reactions between Se and chemical interferents in the matrix of the urine prevented the derivatization of Se. The 4NPD derivatizing reagent is specific towards its reaction with Se (Reamer and

Veillon 1983), thus it is unlikely 4NPD was combining with other elements in the matrix. The measurement of Se in urine was not necessary for quantitation of the endogenous fecal excretion of Se or true absorption and therefore no further attempt was made to measure Se in urine by GCMS.

GCMS analysis should be capable of measuring all six stable isotopes of Se. In the present study the measurement by GCMS of only three of the six stable isotopes of Se was investigated. Using the most abundant isotope of Se as the reference isotope (^{80}Se), Se-76 and Se-78 served satisfactorily as the metabolic tracer and the internal standard respectively. Reamer and Veillon (1983) have also reported the successful application of a method of GCMS analysis based on ^{80}Se as the reference isotope, Se-76 as the metabolic tracer and Se-82 as the internal standard. They reported precision of the measurement, expressed as a relative standard deviation, was 1.4% for 20 determinations of samples of about 40 ng Se mL, and was independent of the sample type (Reamer and Veillon 1983).

In this study ICPMS proved capable of measuring four of the six stable isotopes of Se (^{76}Se, ^{77}Se, ^{78}Se and ^{82}Se). ^{80}Se could not be measured by ICPMS because of interference by Ar_2^+ ion. ^{74}Se was not investigated as the Se-74 isotope preparation costs $100-200 US/mg. The cost of this isotope preparation makes it an unlikely choice as a tracer for experiments with dairy cattle.

Successful application of the stable isotope approach for

the study of Se metabolism depends on the degree of achieved isotope enrichment in relation to the analytical precision with which isotopic measurements can be made. The precision of Se stable isotope enrichment measured by GCMS was better for Se-76 than for Se-78 (Table 7). Thus for experiments involving a single tracer, Se-76 would be the isotope chosen for enrichment and Se-78 for quantitative measurement. The precision obtained for measurement of Se stable isotopes was greater for sample analysis by ICPMS than by GCMS. The best precision obtained from ICPMS analysis (Table 9) was for the measurement of Se-77 and therefore, it would be the isotope of first choice for enrichment purposes. For double isotopic enrichment, Se-77 and Se-82 are the isotopes of choice with Se-76 as the internal standard.

Tracer levels of stable isotopes are typically only a few percent above natural isotopic levels, thus, if stable isotopes are to be used as tracers, their concentration must be determined with high precision. Under the experimental protocol of Trial I the level of precision obtained for GCMS analysis was sufficient to measure Se-76 enrichment in the samples of interest. However a reduction in the quantity of the isotope administered (20 mg) may be necessary under some circumstances. For kinetic analysis it is important to produce a minimum perturbance to the kinetics of a tracer pool and it is advisable to add no more than about 10% of the pool size as tracer (Buckley et al. 1982).
It is also desirable to work with smaller quantities of tracer, such as those administered to the animals in Trial II and III

(Se-77, ~4.6 mg; Se-82, ~1.3 mg), for economic reasons. It may be possible to administer smaller quantities of tracers as the precision of the isotope ratio determination is expected to improve with continued method development of the ICPMS technique. The precision obtained with GCMS analysis however, would not permit the tracer enrichment in the feces to be measured following administration of similar quantities of isotope as in Trial II and III.

5.2. Selenium content of the experimental diets

The dietary concentration of Se has been shown to influence the concentration of ^{75}Se retained by the tissues following both oral and intravenous routes of isotope administration (Lopez et al. 1969). As dietary Se intake increased the specific activity of ^{75}Se in tissues decreased indicating reduced Se retention or increased Se turnover (Lopez et al. 1969; Kincaid et al. 1977).

Trial I was a first attempt at measuring the endogenous fecal excretion and true absorptin of Se and therefore only approximations could be made with regard to the quantity of tracer required to obtain measurable levels of tracer enrichment in the biological samples of interest. Therefore in Trial I it was desirable to provide a low-Se diet to the two cows in order to maximize the uptake and enrichment of the whole body Se pool with the Se-76 tracer.

For Trial II and III the target Se concentration for the experimental diets was 0.2 mg kg^{-1}. The dietary Se concentration

was chosen in anticipation of a proposed revision to the Nutrient Requirements of Dairy Cattle (NAS-NRC 1988) that would increase the recommended Se intake to 0.2 mg kg^{-1}. Selenite was the chemical form of the Se added to the experimental diets as this is the form used by feed companies in British Columbia.

The close association between Se and Vit E in preventing deficiency conditions has been recognized for many animal species (Jenkins and Hidiroglou 1972). The quantity of Vit E considered adequate to meet the dairy cow's requirements lies between 10 and 15 mg kg^{-1} dietary DM (ARC 1980). The Vit E content in orchard grass hay is ~190 mg kg^{-1} (Ensminger and Olentine 1978). Therefore the Vit E consumed by the cows from the diet in this study was considered adequate.

5.3. Tissue distrubution of selenium

In ruminants of an adequate Se status the highest concentration of Se is in the kidney, with the cortex containing a higher concentration than the medulla (Scholz et al. 1981a). High concentrations are also reported in the small intestine, lung, liver, pancreas, adrenal and pituitary glands, and spleen (Kincaid et al. 1977; Scholz et al. 1981a). Handreck and Godwin (1970) report that higher levels of Se are found in the cardiac muscle than in the skeletal muscle. The lowest levels of Se are reported found in the bone, adipose tissue and plasma. The distribution of Se in the tissues and fluids of the two cows in this study were consistent with the results above.

The concentration of Se in the liver and serum have been suggested as useful indicators for the assessment of the Se status of cattle (Thompson et al. 1980, 1981). Based on data from studies involving cattle of adequate Se status (Table 1) the Se concentration of the liver and plasma ranged from 0.80-1.75 $\mu g\ g^{-1}$ and 0.034-0.112 $\mu g\ mL^{-1}$. The mean concentration of Se in the liver was 0.87 and 0.99 $\mu g\ g^{-1}$ and in serum was 0.048 and 0.049 $\mu g\ mL^{-1}$ for the cows of Trial I and Trial II and III, respectively. These values fall within the ranges reported above and indicate the cows at the time of tissue sampling were of an adequate Se status. Had the animals of Trial I continued to consume the low Se diet (0.035 mg kg^{-1}) it is likely their tissue Se levels would have declined to below the normal ranges for Se concentration.

There was a large range of Se-76 enrichment in the tissues and fluids (Fig. 7) as has been reported by other authors for ^{75}Se concentrations (Lopez et al. 1969; Kincaid et al. 1977; Dejneka et al. 1979). Lopez et al. (1969) reported the ^{75}Se concentration in tissues of lambs 12-15 days after ^{75}Se administration in descending order were as follows: kidney, liver, spleen, lung, bile, brain, and heart. In the present study the mean Se-76 enrichment was similar in kidney and liver. The differences in the patterns of tracer distribution in the kidney and liver may be due to to an effect of dietary Se level. In another experiment, Lopez et al. (1969) found lambs fed a ration containing 0.014 mg Se kg^{-1} retained a higher ^{75}Se

concentration in the kidney than in the liver. When lambs received additional Se in the ration (0.264 - 5.014 mg Se kg^{-1}) the retention by these tissues was reversed. Lopez et al. (1969) reported in lambs receiving additional Se, more than 99% of the ^{75}Se activity in the kidney was present in the cortex with a neglegible amount present in the medulla. In the present study Se-76 enrichment in the kidney cortex and medulla were similar. The position of the spleen in the pattern of descending order of tracer enrichment also differed from that reported by Lopez et al. (1969). Se-76 enrichment in descending order was lung, bile, heart and spleen. Lopez et al. (1969) reported accumulations of ^{75}Se in the pelt, gastrocnemius muscle and femur bone were about the same and were higher than in adipose tissue. The enrichement of Se-76 was higher in the hide than in skeletal muscle. Se-76 enrichment was not measurable in bone or adipose tissue as it was below the detection limit for GCMS analysis. The detection limit and therefore the Se-76 enrichment in bone, was less than one-third the level of enrichment in the skeletal muscle. Some of the differences found between the patterns of ^{75}Se and Se-76 distribution may be explained by the age and stage of development of the experimental animals (growing lambs verses mature dairy cows). This was evident from the comparison of Se-76 enrichment of the fetal tissues (Fig. 8) with that of the dam (Fig. 7). The fetal liver and kidney were enriched to levels comparable to those of the corresponding tissues in the dam, however, the enrichment in the fetal muscle was ~6 times the level in the

muscle of the dam.

5.4. Method for measurement of true absorption

The evaluation of the bioavailablity of a nutrient includes an assessment of its availability for intestinal absorption and its subsequent utilization. In monogastric species Se is regarded as well absorbed and the availability for intestinal absorption as essentially complete. These studies have lead to the belief that there is little or no homeostatic regulation of Se at the gastrointestinal level. More recently, studies with experimental animals have focused on monitoring only changes in the availability of Se for incorporation into the biochemically active form of GSHPx in plasma, erythrocytes and occasionally liver and other tissues (Barbezat et al. 1984). In ruminant animals however, the availability of Se for intestinal absorption plays a significant role in the overall availability of this element to the animal. The conditions existing within the rumen tend to reduce the Se to less available chemical forms which pass from the digestive tract via the feces.

The nature of this action and the potential for multiple dietary interactions remain largely unknown. In addition there is a lack of quantitative experimental data on the various aspects of gastrointestinal absorption of Se, including the contribution from endogenous components to fecal Se. Influencing factors on the gastrointestinal absorption and endogenous secretion of Se are of fundamental importance for a more complete

understanding of gastrointestinal function and in relation to dietary management.

Apparent or net absorption determined by conventional balance techniques provides only a rough estimate of absorption. This measure does not make any reference to the origin of the nutrient in the feces and accordingly does not correct for the endogenous fecal loss. Endogenous excretion of many elements makes a considerable contribution to the fecal output and for such elements balance studies underestimate the true extent of absorption. True absorption measures the proportion of a nutrient in food which moves from the intestinal lumen through the mucosal cell and into the body. To measure true absorption the fecal excretion of the nutrient is corrected for the endogenous fecal loss. Endogenous losses of elements via the feces may originate from hepatobiliary transport, secretion by the pancreas and glands of the alimentary tract, sloughing of intestinal epithelial cells and direct secretion from blood into the gastrointestinal tract (Gregus and Klaassen 1986).

In this study quantitation of the endogenous fecal excretion of Se and true absorption were determined following the administration of Se stable isotopes in conjunction with conventional metabolic balance techniques. This procedure has been applied successfully to measure true absorption of Zn with radioisotopes in rats (Evans et al. 1979) and stable isotopes in humans (Jackson et al. 1984).

The endogenous fecal Se was estimated indirectly based on

the tracer enrichment in a reference or index tissue which was assumed to reflect the enrichment of endogenous fecal Se during the balance period. Prospective indices were evaluated by examination of the distribution of the Se-76 in tissues and fluids following a period to allow equilibration of the tracer with natural Se in the body. The length of time required for tracer equilibration will depend upon the element, the route of the isotope administration and the subject species. In rats dosed intramuscularly with radioactive ^{65}Zn, balance periods were begun 9 days following isotope administration (Evans et al. 1979). In the experiment by Jackson et al. (1984) the balance study commenced 2 days subsequent to the intravenous administration of the enriched stable isotope Zn-67 in humans.

The bioeffectiveness and metabolism of trace elements may be influenced by several dietary and host factors. One key factor affecting the availability of Se is the chemical form. In many of the experimental investigations involving the use of tracers to study trace element metabolism the route of isotope administration is via injection. A major underlying assumption is that the administration route does not affect the results. Trace elements, however, enter the animal body from the digestive tract and may undergo transformations during this process. To ensure data obtained from experimental investigations with intravenous administration of the tracer reflects the metabolism of the element under practical situations, the tracer should be administered in the same chemical form as the absorbed element.

In ruminants the chemical form of Se ingested and hence the chemical form of Se absorbed is influenced by the rumen environment. It is not known what the chemical form(s) are of the Se that is absorbed. Thus to ensure the Se-76 tracer would be under similar influences as dietary Se, the tissue distribution of the tracer was determined following oral administration.

Symonds et al. (1981a) reported that the clearance of ^{75}Se from the whole body of dairy cows was described by two exponential components. The first component of clearance consisted of unabsorbed ^{75}Se as well as absorbed ^{75}Se excreted in urine and the gastrointestinal tract with little or no equilibration with the main Se pools of the body. The rentry of the absorbed ^{75}Se into the gastrointestinal tract during this time would not be quantitatively the same as the endogenous secretions as this equivalency does not take place until isotopic equilibration occurs within those body pools responsible for endogenous secretions. At 8 days after oral dosing with ^{75}Se-labelled barley the contribution of ^{75}Se from the first component of clearance was not detectable and the subsequent clearance from the body was considered to be by endogenous loss (Symonds et al. 1981a). Based on this information and the need for a suitable length of time for dietary adaptation, the equilibration period in the present study was 10 (Trail I) and 14 days (Trail II and III).

If equilibration of Se-76 in the tissues was not complete

prior to obtaining balance measurements, Se-76 from the first component of clearance would be expected to contribute to and elevate the total Se-76 enrichment in the feces. The effect of the elevated Se-76 enrichment in feces on the estimation of the endogenous fecal Se would depend also, on the behavior of Se-76 in the index tissue. Langlands et al. (1986) measured ^{75}Se (counts min^{-1} mass Se^{-1}) in feces and plasma collected 1 to 45 days after intravenous administration of $[^{75}Se]SeO_3^{2-}$. Using this data, the calculated ratio of ^{75}Se in the feces to ^{75}Se in plasma, tended to be higher during days 1 to 10. It is suspected there would be a tendancy to overestimate the contribution of the endogenous Se to the total fecal Se and hence underestimate the true absorption of Se if determinations were made from data collected prior to equilibration of the tracer with the body Se.

Twenty-two days following the administration of Se-76 (10 days equilibration, 10 days balance), the tissue and fluids considered as possible contributors to the endogenous fecal Se were found to be enriched to a similar degree. These tissues included the serum, epithelium of the stomach (including the rumen, reticulum, omasum and abomasum), liver, bile, pancreas, small intestine and colon (Fig. 7). The range of enrichment in these tissues (mean of two cows) was TTMP 9.8 to 12.9.

In ruminant animals saliva may also make a considerable contribution to the endogenous secretion of an element. In the present study the enrichment of Se-76 in the salivary glands was not measured. But Dejneka et al. (1979) measured ^{75}Se

concentration in tissues and fluids of sheep and reported a high level of enrichment in the parotid salivary gland.

The liver has been identified as playing a key role in Se metabolim (Symonds et al. 1981a; McMurray and Davidson 1985). Se is believed to be assimilated by the liver where a number of selenocompounds and specific selenoproteins are synthesized and released (McMurray and Davidson 1985). The liver also makes a direct contribution to the fecal Se via bilary Se excretion. It has been hypothesized that one of several of the selenoproteins released by the liver into the plasma act as transport proteins to distribute selenocompounds to other tissues (Motsenbocker and Tappel 1982a; McMurray and Davidson 1985). Thus the serum because of its possible transport function provides a medium for exchange of Se between the tissues including the gastrointestinal tract, liver, pancreas and saliva. The liver and serum play a central and interrelated role in Se metabolism, distribution and excretion and therefore these tissues were believed to best represent the enrichment of the Se tracer of endogenous fecal origin. The use of liver or serum as tissue indices for the calculation of endogenous fecal Se also offer an advantage from a practical standpoint. With the development of routine procedures for obtaining liver biopsy samples and the accessibility of blood, these tissues can be easily sampled.

The effect of the choice of the tissue sampled (liver or serum) on the estimation of endogenous fecal Se excretion and true absorption were evaluated in Trial II and III. There was

close agreement between the level of enrichment measured in serum and liver (Table 21) following oral administration of Se-77 and hence the estimation of endogenous fecal Se from these two indices were equivalent (Table 25).

In addition to the evaluation of the choice of tissue sampled on the estimation of endogenous fecal Se and true absorption, the effect of oral and intravenous routes of tracer administration were also evaluated. The advantage to using the intravenous route of tracer administration is that less isotope is required for tissue enrichment reducing the cost of the experiment.

When Se-82 was administered intravenously a difference was apparent in the degree of enrichment in serum and liver (Table 19). Enrichment in the liver was higher than that measured in the serum. As a result the estimation of endogenous fecal Se following intravenous administration with analysis of the liver was lower than that determined from the analysis of serum. A comparison of the estimates of endogenous fecal Se from oral and intravenous routes of administration with serum and liver analysis revealed all estimates were equivalent except for the estimate determined from enrichment of liver following intravenous administration of the isotope. Major issues related to differential metabolism of Se administered via oral and intravenous routes are still unresolved. However the similarity between the estimates for endogenous fecal Se from the oral route of isotope administration with the analysis of serum or liver and

the intravenous route of isotope administration with the analysis of serum indicate that if differential metabolism exists, it does not affect the determination of endogenous fecal Se by these methods. There does however, appear to be differences in the metabolism of Se by the liver depending on the route of administration. Therefore, for estimation of endogenous fecal Se, the oral route of tracer administration with the analysis of serum or liver and the intravenous route of tracer administration with the analysis of serum were found suitable.

The absolute values and the associated variation for the daily Se intake and the total fecal Se were large relative to that for the estimation of endogenous fecal Se. As a result apparent absorption of Se varied widely among animals and when corrected for the contribution of endogenous Se in the feces to calculate the true absorption, differences among the 4 estimates of true absorption were not statistically significant (Table 26). Thus it would appear true absorption of Se could be determined from the oral or intravenous route of isotope administration with analysis of serum or liver. The values for the 4 estimates of true absorption however, do show a trend similar to the values for the estimate of endogenous fecal Se. That is, the estimates of true absorption determined from the oral route of tracer administration with serum and liver analysis and from the intravenous route of administration with serum analysis were more closely comparable and the estimate from the intravenous route of administration with liver analysis was lower. If circumstances

prevailed where the endogenous fecal Se loss contributed a greater portion to the overall fecal Se excretion (such as in Trial I (Table 15) where the animals were consuming a low Se diet), it is expected significant differences between the 4 estimates for true absorption would parallel that for the 4 estimates of endogenous fecal Se. Therefore it is recommended that true absorption of Se be determined from one of the approaches suggested for determination of endogenous fecal Se loss: i.e. the oral route of tracer administration with the analysis of serum or liver or the intravenous route of tracer administration with the analysis of serum.

5.5. Apparent and true absorption of selenium

Harrison and Conrad (1984a) measured apparent absorption of Se in non-lactating dairy cows with dietary Se intakes ranging from 437 to 3136 $\mu g\ d^{-1}$. The quantity of Se absorbed ($\mu g\ d^{-1}$) increased with increasing Se intake and was described by the linear relationship: $Y = 0.51X - 132$, $r^2 = 0.98$. Extrapolation of the linear regression line to zero absorption indicated cows consuming below 259 $\mu g\ d^{-1}$ would be in a negative Se absorptive state. In Trial I the two cows were found to be in a negative Se absorptive state when consuming 360 - 460 μg Se d^{-1}. The practical significance of the data from these two animals and that from Harrison and Conrad (1984a) suggested that non-lactating cows consuming a diet with a Se concentration $\leq$ 0.035 mg kg^{-1} DM would be in a negative Se balance. Therefore,

it is important for non-lactating pregnant cows consuming low Se diets to receive Se supplementation at a time when adequate provision of Se is necessary for the reproductive health of the cow (Harrison and Conrad 1984a).

Apparent absorption and true absorption of Se by non-lactating cows consuming a diet with a Se concentration of 0.19 mg kg^{-1} was 3% (Table 22) and 11% (Table 26) of the Se intake (2600 μg d^{-1}), respectively. Harrison and Conrad (1984a) have reported much higher values of 46% and 50% for apparent absorption by cows consuming 2701 and 3136 μg Se d^{-1}. Several factors may be responsible for the disparity between the results reported here and those of Harrison and Conrad (1984a). These include the source of Se and possible interactions arising between Se and other dietary components. Work by Peter et al. (1982, cited by Peter et al. 1985) indicated organic Se was absorbed to a greater extent than inorganic Se. These authors reported sheep of a low Se status fed a low Se diet and infused intraruminally with SeO_3^{2-} or Se-Met, absorbed 12% to 13% more Se from Se-Met than SeO_3^{2-}. The major Se compounds in seeds or forages consumed by livestock are organic and include Se-Met, Se-Cys, selenocystine and Se-methylselenomethionine (NAS-NRC 1983). The Se in the rations of the study by Harrison and Conrad (1984a) were provided all or in a large part by natural sources and therefore, the Se was primarily in an organic form. In trial II and III the Se was provided primarily as selenite, an inorganic form. However, a comparison of the results for true

absorption of Se from the cows of Trial II and III (11%) which consumed primarily selenite, with the results from the two cows of Trial I (10% and 16%) which received Se from a natural source, suggested the absorption of Se from feedstuffs and selenite were similar.

Differences in the composition of the diets in the study of Harrison and Conrad (1984a) and the present study, resulting in differences in possible interactions among Se and other dietary factors may also be involved. Harrison and Conrad (1984b) reported a maximum Se absorption in cows of 40% to 50% when the dietary Ca was 0.8% of dry matter intake. At dietary Ca intakes of 0.4% to 0.5% of dry matter intake, Se absorption decreased by ~20 %. The concentration of Ca in this study was 0.48%, and therefore, might have contributed to the relatively low rates of absorption.

Selenite has been reported to be less stable than the other common form of inorganic Se, selenate (Ammerman and Miller 1974). The reduction of selenite to elemental Se forms may be observed upon exposure to organic matter. This can cause practical problems when selenite is incorporated into diets as a premix with reducing sugars (Ganther 1984). Molasses contains $\geq$ 46% total sugars (Church 1984), however, these sugars exist primarily as sucrose which is a non-reducing sugar. Thus, reduction of selenite to the less available elemental form upon its addition to molasses was not believed to be a contributing factor towards the low values for Se absorption found in this study.

5.6. Selenium excretion

Butler and Peterson (1961) reported that the main pathway of Se excretion in ruminants is via the feces when dietary Se levels are below 0.01 mg kg^{-1}. When Se supply is adequate Se is excreted via the urine and feces in about equal proportions (Lopez et al. 1969). As the Se supply is increased the urinary excretion of Se also increases and may even exceed fecal Se excretion at high levels of administration. In the present study a greater proportion of the dietary Se intake was excreted in the feces. The excretion of Se in the feces constituted ~90% of the total daily Se excreted, with the remainder of Se excreted in the urine (Trial II and III, Table 22). The endogenous fecal Se was 9.7% of the total fecal Se (Trial II and III, Table 23). In cows consuming a low Se diet (0.035 mg kg^{-1}), the contribution of endogenous Se to the total fecal Se was increased to 23% and 36% (Table 15). The excretion of Se in expired air was considered to be insignificant and was not measured in this experiment. Handreck and Godwin (1970) reported sheep receiving 0.5 to 1.3 mg Se d^{-1} excreted only about 1% in expired air.

There is very little information regarding the quantity of Se contributed by the various sources to the endogenous fecal Se pool. In sheep dosed intravenously with [^{75}Se]Se-Met, the radioactivity 28 d post injection in the anterior section of the small intestine was ~100 times that in the feces suggesting a large flow of endogenous Se into the small intestine (Langlands et al. 1986). In another experiment, Langlands et al. (1986)

reported approximately 300 to 400 μg Se entered the anterior portion of the small intestine of sheep each day; equal to two to three times the quantity of Se ingested, most of which was subsequently reabsorbed.

Gregus and Klaassen (1986) administered ^{75}Se intravenously to rats and by comparison of the bilary and fecal Se excretions were able to predict whether the Se was excreted or reabsorbed by the intestine. The 2-h bilary excretion was higher than the 4-d fecal excretion suggesting Se underwent intestinal reabsorption. Langlands et al. (1986) reported the Se concentration in bile of sheep averaged 0.0086 μg Se mL^{-1}, which represented a daily excretion of 13.8 μg or 28% of the Se consumed. In this study the excretion of Se from bile into the gastrointestinal tract based on an estimated bilary secretion of 525 mL h^{-1} (Symonds et al. 1981b) and a bilary Se concentration of 0.0061 μg mL^{-1} (Trial I) was 769 μg d^{-1}. This is 1.5 times the the daily fecal Se excretion and also suggests reabsorption is taking place within the intestine. Recycling of Se would not have affected the estimation of endogenous fecal Se in the present study as the method described herein measured the net amount of unabsorbed Se of endogenous origin in the fecal pool.

5.7. Effect of copper on the endogenous fecal excretion and true absorption of selenium

Major economic losses in animal production may arise from deficiencies or imbalances in trace element intake. This becomes

particularly important with regard to Se where problems exist in the diagnosis of subclinical deficiency. Further problems are evident in defining the animals dietary requirements, as situations under practical conditions have developed where animals respond to Se supplementation despite receiving what was considered adequate. The involvement of many factors in the Se/Vit E responsive conditions seem apparent from the wide variation in what is reported to be selenium deficiency in cattle (Fenimore et al. 1983). Fenimore et al. (1983) reported beef cattle in southeastern British Columbia fed moderate to low Se levels in hay responded to Se supplementation with the disappearance of a variety of clinical observations including weak calves, neonatal diarrhea, pneumonia, poor growth, placental retention and poor reproductive performance. Fundamental quantitative data are necessary for a comprehensive understanding of Se metabolism and the complex series of host and dietary interactions which are likely to be involved in influencing the Se economy.

Selenium and sulfur show similar chemical characteristics and Se shares with S an affinity for heavy metals such as cadmium, silver, mercury and copper. An increase in the intake of S, either as sulfate or an organic form can reduce liver and blood Cu concentrations in sheep. It is believed that Cu absorption is decreased through an interaction in the alimentary tract, possibly involving the formation of S^{2-} in the rumen and the formation of insoluble CuS, a relatively non-available form

of Cu (Bremner and Davies 1980). The chemical environment in the rumen would probably lead to the formation of Se^{2-} and the formation of metal selenides (Peterson and Spedding 1963). A parallel between the formation of CuS and the formation of insoluble CuSe likely exists within the rumen. Therefore it was hypothesized that an interaction between Cu and Se in the rumen might result in the formation of CuSe and thereby reduce the availability of Se for absorption.

Interaction between Cu and inorganic Se have been demonstrated in chicks (Hill 1974; Jensen 1975a) where high dietary Cu (500 - 1000 mg kg^{-1}) was found to counteract Se toxicity. High dietary Cu concentrations (800 - 1600 mg kg^{-1}) have also been reported to induce Se deficiency in chicks (Jensen 1975a) and ducklings (Van Vleet and Boon 1980). It was suggested that Cu formed insoluble complexes with Se within the gastrointestinal tract and tissues thereby reducing the availability of Se. In ruminants, high levels of Cu supplementation were found to increase the apparent Se retention (White et al. 1979; Gooneratne et al. 1981). Gooneratne et al. (1981) reported the accumulation of Cu in the liver of copper loaded sheep was associated with an increase in liver Se concentration and GSHPx activity. It was suggested that increased Se retention in sheep was a response to tissue damage caused by Cu accumulation (White et al. 1979). Thus there appears to be a metabolic interaction between Se and Cu when excessively high concentrations of Cu are used. It is however,

less clear whether the interactions between Cu and Se have significant metabolic or pathological consequences when Cu concentrations are more typical of those incurred in practical situations arising from variations of supplemental Cu added to the diet.

Abdel Rahim et al. (1986) investigated the effect of lower concentrations of dietary Cu (1.3 - 200 mg kg^{-1}) on Se utilization by the rat. Cu supplementation did not affect absorption and total-body retention of ^{75}Se from $[^{75}Se]SeO_3^{2-}$. Only when the dietary Cu concentration reached 200 mg kg^{-1} was there an influence on the organ distribution of ^{75}Se in tissues. This occurred when ^{75}Se was administered orally and intraperitoneally. There was a reduction in the Se concentration in liver, kidneys and whole blood and reduced GSHPx activities in liver, testis, kidney and whole blood. Increasing the Cu concentration of the diets did not result in significantly higher Cu concentrations in the tissues and it was suggested that the 200 mg Cu kg^{-1} diet may have resulted in the appearance of a novel Se-reactive form of Cu or a higher rate of turnover of an existing reactive pool of Cu (Abdel Rahim et al. 1986).

Fehrs et al. (1981) studied the effects of supplementing diets with 0 and 100 mg Cu kg^{-1} on the Se metabolism in calves. Calves were fed diets containing 0 or 1.0 mg Se kg^{-1}. Forty-eight hours following an oral dose of ^{75}Se, ^{75}Se in blood, kidney and spinal cord was lower, and more ^{75}Se was excreted in the urine and feces of calves receiving the high Cu and Se diets,

indicating a lower ^{75}Se retention. In contrast supplementation of the low Se diet with 100 mg Cu kg^{-1} did not alter the excretion of ^{75}Se in feces and urine or the distribution of ^{75}Se. White et al. (1981) also reported no significant effect of supplemental Cu (10 mg kg^{-1}) on the absorption or the excretion and tissue retention of ^{75}Se 7 days following the oral administration of [^{75}Se]Se-Met. In the present study with dairy cattle offered a practical diet, there was no significant effect of 17 mg kg^{-1} of supplemental Cu on the excretion and true absorption of Se. In addition Cu supplementation did not influence the distribution of Se-77 and Se-82 in serum or liver. These results are in agreement with Fehrs et al. (1981) and White et al. (1981) and suggest there is no significant Cu-Se interaction or metabolic consequence arising when the dietary concentrations of Se and Cu are typical of those found under practical conditions.

6. CONCLUSIONS

1. ICPMS and GCMS both proved to be accurate for quantitative analysis of Se by isotope dilution as indicated by good agreement between measured and certified Se values in standard reference materials (Table 6 and 8).

2. GCMS was found to be suitable for the measurement of Se-76 and Se-78 enrichment and ICPMS for the measurement of Se-76, Se-77 and Se-82 enrichment. The isotopes of choice for metabolic tracers were Se-76 when samples were analyzed by GCMS and Se-77 and Se-82 when analyzed by ICPMS.

3. Greater precision for measurement of Se stable isotope enrichment in biological materials was obtained for ICPMS than for GCMS (Table 7 and 9). The advantage to using the most precise method of analysis is that a smaller quantity of the tracer may be administered to the dairy cow thereby minimizing the possibility of altering Se metabolism as well as reducing the cost of the experiment.

4. The combination of conventional metabolic balance techniques with isotopic enrichment of the body Se pools was considered to yield reliable estimates of the endogenous fecal excretion of Se and true absorption of Se in dairy cows. The reliability of the method was evaluated by investigation of the enrichment of Se

pools which were potential contributors to the endogenous fecal Se and by studying the effect of route of administration of the tracer. The application of this technique using Se stable isotopes as tracers will enable further experimental investigations to increase the understanding of ways in which the efficiency of Se absorption may vary and thereby further improve the ability to estimate the dietary requirements for Se in dairy cattle.

5. Tracer enrichment was similar (TTMP 9.8 to 12.9) in tissues considered to be potential contributors to the endogenous fecal Se (serum, epithelium of the stomach, liver, bile, pancreas, small intestine and colon).

6. Serum and liver were selected as tissue indices for the calculation of endogenous fecal Se and true Se absorption. The selection of the index tissue was dependent on the route of tracer administration. To ensure an accurate measurement of endogenous fecal Se and true absorption of Se under a variety of conditions it is recommended these estimates be made from the analysis of serum or liver with an oral route of tracer administration or from analysis of serum with an intravenous route of administration.

7. In dairy cattle consuming a Se-supplemented diet containing 0.19 mg Se kg^{-1} (primarily as selenite) apparent absorption and

true absorption was 3.2 and 11% of Se intake, respectively. In two cows fed a Se deficient diet (0.035 mg kg^{-1}) apparent absorption of Se was negative (-8 and -41%).

8. In dairy cows consuming the Se-supplemented diet the percentage of the fecal Se of endogenous origin was 9.7%. In the cows fed the Se-deficient diet the fecal Se of endogenous origin was higher and equal to 23 and 36% of the total fecal Se.

9. There was no effect of supplemental dietary Cu (17 mg kg^{-1}) on the endogenous fecal Se excretion or the true absorption of Se in dairy cows. Thus there appears to be no Cu-Se interaction occurring in the gastrointestinal tract which would cause a reduction of the absorption of Se supplemented as sodium selenite.

7. BIBLIOGRAPHY

Abdel Rahim, A. G., Arthur, J. R. and Mills, C. F. 1986. Effects of dietary copper, cadmium, iron, molybdenum and manganese on selenium utilization by the rat. J. Nutr. 116: 403-411.

Agriculture Research Council. 1980. Pages 249-250 in The nutrient requirements of livestock. ARC, Commonwealth Agricultural Bureaux, Slough, United Kingdom.

Ammerman, C. B. and Miller, S. M. 1974. Selenium in ruminant nutrition: a review. J. Dairy Sci. 58: 1561-1577.

Association of Official Analytical Chemists. 1980. Official methods of analysis. 13th ed. AOAC, Washington, D.C.

Barbezat, G. O., Casey, C. E., Reasbeck, P. G., Robinson, M. F. and Thomson, C. D. 1984. Selenium. Pages 231-258 in N. W. Solomons and I. H. Rosenberg, eds. Absorption and malabsorption of mineral nutrients. Alan R. Liss, Inc., New York.

Behne, D., Hofer-Bosse, T. and Elger, W. 1987. Selenium and hormones in the male reproductive system. Pages 733-739 in G. F. Combs, Jr., O. A. Levander, J. E. Spallholz and J. E. Oldfield, eds. Selenium in biology and medicine. Part B. Van Nostrand Reinhold Co., New York.

Beilstein, M. A., Tripp, M. J. and Whanger, P. D. 1981. Evidence for selenocysteine in ovine tissue organelles. J. Inorg. Biochem. 15: 339-347.

Beilstein, M. A., Butler, J. A. and Whanger, P. D. 1984. Metabolism of ^{75}Se-selenite by rhesus monkeys. J. Nutr. 114: 1501-1509.

Beilstein, M. A. and Whanger, P. D. 1986. Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. J. Nutr. 116: 1711-1719.

Beilstein, M. A. and Whanger, P. D. 1987. Distribution of selenium in erythrocyte fractions of humans and animals. Pages 265-277 in G. F. Combs, Jr., O. A. Levander, J. E. Spallholz and J. E. Oldfield, eds. Selenium in biology and medicine. Part A. Van Nostrand Reinhold Co., New York.

Boyne, R. and Arthur, J. R. 1979. Alterations of neutrophil function in selenium deficient cattle. J. Comp. Pathol. 89: 151-158.

Bremner, I. and Davies, N. T. 1980. Dietary composition and absorption of trace elements by ruminants. Pages 409-427 in Y. Ruckebusch and P. Thivend, eds. Digestive physiology and metabolism in ruminants. MTP Press Ltd., Lancaster, England.

Brown, D. G. and Burk, R. F. 1973. Selenium retention in tissues and sperm of rats fed a torula yeast diet. J. Nutr. 103: 102-108.

Buckley, W. T., Huckin, S. N. and Budac, J. J. 1982. Mass spectrometric determination of a stable tracer for copper in biological materials. Anal. Chem. 54: 504-510.

Buckley, W. T., Eigendorf, G. K. and Dorward, W. J. 1986. A liver biopsy instrument for large animals. Can. J. Anim. Sci. 66: 1137-1140

Buckley, W. T., Godfrey, D. V., Koenig, K. M. and Shelford, J. A. 1987. Determination of selenium and multiple stable isotope enrichment of selenium in biological materials by inductively coupled plasma mass spectrometry. In L. S. Hurley, C. L. Keen, B. Lonnerdal and R. B. Rucker, eds. Trace elements in man and animals - TEMA 6. Plenum Publishing Co., New York. In press.

Buckley, W. T. 1987. The use of stable isotopes in studies of mineral metabolism. Proc. Nutr. Soc. In press.

Butler, G. W. and Peterson, P. J. 1961. Aspects of fecal excretion of selenium by sheep. N. Z. J. Agric. Res. 4: 484-491.

Calvin, H. I., Cooper, G. W. and Wallace, E. 1981. Evidence that selenium in rat sperm is associated with a cysteine-rich structural protein of the mitochondrial capsule. Gamete Res. 4: 139-149.

Cardin, C. J. and Mason, J. 1975. Sulphate transport by rat ileum. Effect of molybdate and other anions. Biochim. Biophys. Acta. 394: 46-54.

Cary, E. E., Allaway, W. H. and Miller, M. 1973. Utilization of different forms of dietary selenium. J. Anim. Sci. 36: 285-292.

Cathcart, E. B., Shelford, J. A. and Peterson, R. G. 1980. Mineral analyses of dairy cattle feed in the Upper Fraser Valley of British Columbia. Can. J. Anim. Sci. 60: 177-183.

Church, D. C. 1984. Pages 123-126 in Livestock feeds and feeding. 2nd ed. O and B Books, Inc., Corvallis, Oregon.

Combs, G. F., Jr. and Combs, S. B. 1984. The nutritional biochemistry of selenium. Ann. Rev. Nutr. 4: 257-280.

Conrad, H. R. 1985. The role of selenium and vitamin E in bovine reproduction. Pages 26-30 in Selenium responsive diseases in food animals. Veterinary Learning Systems Co., Inc., United States.

Cousins, F. B. and Cairney, I. M. 1961. Some aspects of selenium metabolism in sheep. Aust. J. Agric. Res. 12: 927-943.

Crutchfield, W. O. 1968. A technique for placement of an indwelling catheter in the cow. Vet. Med./Sm. Anim. Clin. Dec. pp. 1141-1144.

Davidson, W. B. and McMurray, C. H. 1987. Definition of plasma selenium proteins using two-dimensional electrophoresis. Pages 278-282 in G. F. Combs, Jr., O. A. Levander, J. E. Spallholz and J. E. Oldfield, eds. Selenium in biology and medicine. Part A. Van Nostrand Reinhold Co., New York.

Dejneka, J., Nowosad, R. and Simoni, J. 1979. Badania nad rozmieszczeniem radioselenu ^{75}Se w tkankach i tresci zwacza oraz jego wydalaniem z zolcia, moczem i kalem u owiec. Polskie Archiwum Weterynaryjne 21: 237-248.

Dilworth, G. L. and Bandurski, R. S. 1977. Activation of selenate by adenosine-5'-triphosphate sulfurylase from Saccharomyces cerevisiae. Biochem. J. 163: 521-529.

Diplock, A. T. 1984. Vitamin E, selenium, and free radicals. Med. Biol. 62: 78-80.

Diplock, A. T. 1985. The role of selenium in the control of oxygen-induced cellular damage. Nutr. Res. (Suppl. I): 175-179.

Diplock, A. T. 1987. Metabolic defences against oxygen and xenobiotic toxicity: biochemical interrelationships. Pages 90-103 in G. F. Combs, Jr., O. A. Levander, J. E. Spallholz and J. E. Oldfield, eds. Selenium in biology and medicine. Part A. Van Nostrand Reinhold Co., New York.

Doyle, J. 1979. Toxic and essential elements in bone - a review. J. Anim. Sci. 49: 482-497.

Ensminger, M. E. and Olentine, C. G., Jr. 1978. Pages 1256-1257 in Feeds and nutrition - complete. Ensminger Publishing Co., Clovis, California.

Epp, O., Ladenstein, R., and Wendel, A. 1983. The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. Eur. J. Biochem. 133: 51-69.

Esaki, N., Nakamura, T., Tanaka, H. and Soda, K. 1982. Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. J. Biol. Chem. 257: 4386-4391.

Evans, G. W., Johnson, E. C. and Johnson, P. E. 1979. Zinc absorption in the rat determined by radioisotope dilution. J. Nutr. 109: 1258-1264.

Fehrs, M. S., Miller, W. J., Gentry, R. P., Neathery, M. W., Blackmon, D. M. and Heinmiller, S. R. 1981. Effect of high but non toxic dietary intake of copper and selenium on metabolism in calves. J. Dairy Sci. 64: 1700-1706.

Fenimore, R. L., Adams, D. S. and Puls, R. 1983. Selenium levels of beef cattle in southeastern British Columbia relative to supplementation and type of pasture. Can. Vet. J. 24: 41-45.

Forstrom, J. W., Zakowski, J. J. and Tappel, A. L. 1978. Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochem. 17: 2639-2644.

Ganther, H. E., Levander, O. A. and Baumann, C. A. 1966. Dietary control of selenium volatilization in the rat. J. Nutr. 88: 55-60.

Ganther, H. E. 1975. Selenoproteins. Chemica Scripta 8A: 79-84.

Ganther, H. E. 1984. Selenium metabolism and function in man and animals. Pages 3-24 in Trace element analytical chemistry in medicine and biology. Vol. 3. Walter de Gruyter and Co., Berlin.

Ganther, H. E. 1987. Chemistry and metabolism of selenium. Pages 53-65 in G. F. Combs, Jr., O. A. Levander, J. E. Spallholz and J. E. Oldfield, eds. Selenium in biology and medicine. Part A. Van Nostrand Reinhold Co., New York.

Gasiewicz, T. A. and Smith, J. C. 1978. The metabolism of selenite by intact rat erythrocytes in vitro. Chem. Biol. Interact. 21: 299-313.

Gooneratne, S. R. and Howell, J. McC. 1981. Selenium in copper toxicity in sheep. Pages 468-470 in J. M. Gawthorne, J. McC. Howell and C. L. White, eds. Trace elements in man and animals - TEMA 4. Springer-Verlage, New York.

Grace, N. D. and Watkinson, J. H. 1985. The distribution and amounts of Se associated with various tissues and liveweight gains of grazing sheep. Pages 490-493 in C. F. Mills, I. Bremner and J. K. Chesters, eds. Trace elements in man and animals - TEMA 5. Commonwealth Agricultural Bureaux, Slough, United Kingdom.

Gregus, Z. and Klaassen, C. D. 1986. Disposition of metals in rats: a comparative study of fecal, urinary and bilary excretion and tissue distribution of eighteen metals. Tox. Appl. Pharmacol. 85: 24-38.

Griffiths, N. M., Stewart, R. D. H. and Robinson, M. F. 1976. The metabolism of [^{75}Se]selenomethionine in four women. Br. J. Nutr. 35: 373-382.

Halliwell, B. and Gutteridge, J. M. C. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219: 1-14.

Handbook of chemistry and physics, 51st ed. 1970. R. C. Weast, ed. Chemical Rubber Co., Ohio.

Handreck, K. A. and Godwin, K. O. 1970. Distribution in the sheep of selenium derived from ^{75}Se-labelled ruminal pellets. Aust. J. Agric. Res. 21: 71-84.

Harrison, J. H., Hancock, D. D. and Conrad, H. R. 1984. Vitamin E and selenium for reproduction of the dairy cow. J. Dairy Sci. 67: 123-132.

Harrison, J. H. and Conrad, H. R. 1984a. Effect of selenium intake on selenium utilization by the nonlactating dairy cow. J. Dairy Sci. 67: 219-223.

Harrison, J. H. and Conrad, H. R. 1984b. Effect of dietary calcium on selenium absorption by the nonlactating dairy cow. J. Dairy Sci. 67: 1860-1864.

Hawkes, W. C., Lyons, D. E. and Tappel, A. L. 1982. Identification of a selenocysteine-specific aminoacyl transfer RNA from rat liver. Biochim. Biophys. Acta. 699: 183-191.

Hawkes, W. C., Wilhelmsen, E. C. and Tappel, A. L. 1985. Abundance and tissue distribution of selenocysteine-containing proteins in the rat. J. Inorg. Biochem. 23: 77-92.

Hidiroglou, M., Heaney, D. P. and Jenkins, K. J. 1968. Metabolism of inorganic selenium in rumen bacteria. Can. J. Physiol. Pharmacol. 46: 229-232.

Hidiroglou, M. and Jenkins, K. J. 1972. Le sort du radioselenium administre dans le rumen ou dans la caillette du mouton. Ann. Biol. Anim. Bioch. Biophys. 12: 599-616.

Hidiroglou, M. and Jenkins, K. J. 1973a. Absorption of ^{75}Se-selenomethionine from the rumen of sheep. Can. J. Anim. Sci. 53: 345-347.

Hidiroglou, M. and Jenkins, K. J. 1973b. Fate of ^{75}Se-selenomethionine in the gastrointestinal tract of sheep. Can. J. Anim. Sci. 53: 527-536.

Hidiroglou, M., Jenkins, K. J. and Knipfel, J. E. 1974. Metabolism of selenomethionine in the rumen. Can. J. Anim. Sci. 54: 325-330.

Hill, C. H. 1974. Reversal of selenium toxicity in chicks by mercury, copper and cadmium. J. Nutr. 104: 593-598.

Hill, C. H. 1975. Interrelationships of selenium with other trace elements. Fed. Proc. 34: 2096-2100.

Humaloja, T. and Mykkanen, H. M. 1986. Intestinal absorption of ^{75}Se-labeled sodium selenite and selenomethionine in chicks: effect of time, segment, selenium concentration and method of measurement. J. Nutr. 116: 142-148.

Jackson, M. J., Jones, D. A., Edwards, H. T., Coleman, M. L. and Swainbank, I. G. 1984. A new stable isotope technique for the measurement of zinc absorption and gastrointestinal excretion in man. Nutr. Res. (Suppl. I): 88-91.

Janghorbani, M., Ting, B. T. G. and Young, V. R. 1981. Use of stable isotopes of selenium in human metabolic studies: development of analytical methodology. Am. J. Clin. Nutr. 34: 2816-2830.

Janghorbani, M., Christensen, M. J., Nahapetian, A. and Young, V. R. 1982a. Selenium metabolism in healthy adults: quantitative aspects using stable isotope $^{74}SeO_3^{2-}$. Am. J. Clin. Nutr. 35: 647-654.

Janghorbani, M., Ting, B. T. G., Nahapetian, A. and Young, V. R. 1982b. Conversion of urinary selenium to selenium(IV) by wet oxidation. Anal. Chem. 54: 1188-1190.

Janghorbani, M. 1984. Stable isotopes in nutrition and food science. Progress Food Nutr. Sci. 8: 303-332.

Janghorbani, M., Young, V. R. and Ehrenkranz, R. A. 1985. Isotopic methods in the study of mineral metabolism of infants with special reference to stable isotopes. Pages 63-85 in R. K. Chandra, ed. Trace elements in nutrition of children. Nestle Nutrition, Vevey/Raven Press, New York.

Jenkins, K. J. and Hidiroglou, M. 1972. A review of selenium/vitamin E responsive problems in livestock: a case for selenium as a feed additive in Canada. Can. J. Anim. Sci. 52: 591-620.

Jenkins, K. J., Hidiroglou, M., Wauthy, M. and Proulx, J. E. 1974. Prevention of nutritional muscular dystrophy in calves and lambs by selenium and vitamin E additions to the maternal mineral supplement. Can. J. Anim. Sci. 54: 49-60.

Jensen, L. S. 1975a. Modification of a selenium toxicity in chicks by dietary silver and copper. J. Nutr. 105: 769-755.

Jensen, L. S. 1975b. Precipitation of a selenium deficiency by high dietary levels of copper and zinc (38754). Proc. Soc. Exp. Biol. Med. 149: 113-116

Julien, W. E., Conrad, H. R., Jones, J. E. and Moxon, A. L. 1976a. Selenium and vitamin E and incidence of retained placenta in parturient dairy cows. J. Dairy Sci. 59: 1954-1959.

Julien, W. E., Conrad, H. R. and Moxon, A. L. 1976b. Selenium and vitamin E and incidence of retained placenta in parturient dairy cows. II. Prevention in commercial herds with prepartum treatment. J. Dairy Sci. 59: 1960-1962.

Kaneko, J. J., Zinkle, J. G. and Keeton, K. S. 1971. Erythrocyte porphyrin and erythrocyte survival in bovine erythropoietic porphyria. Am. J. Vet. Res. 32: 1981-1985.

Kincaid, R. L., Miller, W. J., Neathery, M. W., Gentry, R. P. and Hampton, D. L. 1977. Effect of added dietary selenium on metabolism and tissue distribution of radioactive and stable selenium in calves. J. Anim. Sci. 44: 147-151.

Kiremidjian-Schumacher, L. and Stotzky, G. 1987. Reveiw. Selenium and immune response. Environ. Res. 42: 277-303.

Langlands, J. P., Bowles, J. E., Donald, G. E., Smith, A. J., Paull, D. R. and Davies, H. I. 1982. Deposition of copper, manganese, selenium and zinc in the ovine foetus and associated tissues. Aust. J. Agric. Res. 33: 591-605.

Langlands, J. P., Bowles, J. E., Donald, G. E. and Smith, A. J. 1986. Selenium excretion in sheep. Aust. J. Agric. Res. 37: 201-209.

Lawrence, R. A., Sunde, R. A., Schwartz, G. L. and Hoekstra, W. G. 1974. Glutathione peroxidase activity in rat lens and other tissues in relation to dietary selenium intake. Exp. Eye Res. 18: 563-569.

Levander, O. A. 1987. A global view of human selenium nutrition. Ann. Rev. Nutr. 7: 227-250.

Lopez, P. L., Preston, R. L. and Pfander, W. H. 1969. Whole-body retention, tissue distribution and excretion of selenium-75 after oral and intravenous administration in lambs fed varying selenium intakes. J. Nutr. 97: 123-132.

Matthews, C. A., Swett, W. W. and McDowell, R. E. 1975. External form and internal anatomy of holsteins and jerseys. J. Dairy Sci. 58: 1453-1475.

McConnell, K. P. and Cho, G. J. 1965. Transmucosal movement of selenium. Am. J. Physiol. 208: 1191-1195.

McConnell, K. P. and Roth, D. M. 1966. Respiratory excretion of selenium. Proc. Soc. Exp. Biol. Med. 123: 919-921.

McConnell, K. P. and Cho, G. J. 1967. Active transport of selenium in the everted intestine of the hamster. Pages 329-343 in O. H. Muth, J. E. Oldfield and P. H. Weswig, eds. Selenium in biomedicine. AVI Publishing Co., Inc., Westport, Connecticut.

McMurray, C. H. and Davidson, W. B. 1985. Towards a selenium model - time course of selenium binding to plasma and red cells in sheep blood after intravenous administration of ^{75}Se selenite. Pages 474-480 in C. F. Mills, I. Bremner and J. K. Chesters, eds. Trace elements in man and animals - TEMA 5. Commonwealth Agricultural Bureaux, Slough, United Kingdom.

Miltimore, J. E., Ryswyk, A. L., Pringle, W. L., Chapman, F. M. and Kalnin, C. M. 1975. Selenium concentrations in British Columbia forages, grains and processed feeds. Can. J. Anim. Sci. 55: 101-111.

Moksnes, K. and Norheim, G. 1983. Selenium and glutathione peroxidase levels in lambs receiving feed supplemented with sodium selenite or selenomethionine. Acta. Vet. Scand. 24: 45-58.

Motsenbocker, M. A. and Tappel, A. L. 1982a. Selenocysteine-containing proteins from rat and monkey plasma. Biochim. Biophys. Acta. 704: 253-260.

Motsenbocker, M. A. and Tappel, A. L. 1982b. A selenocysteine-containing selenium-transport protein in rat plasma. Biochim. Biophys. Acta. 719: 147-153.

Nahapetian, A. T., Janghorbani, M. and Young, V. R. 1983. Urinary trimethylselenonium excretion by the rat. J. Nutr. 113: 401-411.

National Academy of Sciences - National Research Council. 1978. Nutrient requirements of dairy cattle. 5th ed. NAS-NRC, Washington, D.C.

National Academy of Sciences - National Research Council. 1983. Selenium in nutrition. NAS-NRC, Washington, D.C.

National Academy of Sciences - National Research Council. 1988. Nutrient requirements of dairy cattle. 6th ed. NAS-NRC, Washington, D.C. In press.

Oh, S. H., Ganther, H. E. and Hoekstra, W. G. 1974. Selenium as a component of glutathione peroxidase isolated from ovine erythrocytes. Biochem. 13: 1825-1829.

Oh, S. H., Pope, A. L. and Hoekstra, W. G. 1976a. Glutathione peroxidase response to selenium intake in lambs fed a torula yeast-based artificial milk. J. Anim. Sci. 42: 977-983.

Oh, S. H., Pope, A. L. and Hoekstra, W. G. 1976b. Dietary selenium requirement of sheep fed a practical-type diet as assessed by tissue glutathione peroxidase and other criteria. J. Anim. Sci. 42: 984-992.

Palmer, I. S., Fischer, D. D., Halverson, A. W. and Olson, O. E. 1969. Identification of a major selenium excretory product in rat urine. Biochem. Biophys. Acta. 177: 336-332.

Palmer, I. S., Gunsalus, R. P., Halverson, A. W. and Olson, O. E. 1970. Trimethylselenonium ion as a general excretory product from selenium metabolism in the rat. Biochim. Biophys. Acta. 208: 260-266.

Patterson, E. L., Milstrey, R. and Stokstad, E. L. R. 1957. Effect of selenium in preventing exudative diathesis in chicks. Proc. Soc. Exp. Biol. Med. 95: 617-620.

Paulson, G. D., Baumann, C. A. and Pope, A. L. 1966. Fate of a physiological dose of selenate in the lactating ewe: effect of sulfate. J. Anim. Sci. 25: 1054-1058.

Paulson, G. D., Baumann, C. A. and Pope, A. L. 1968. Metabolism of ^{75}Se-selenite, ^{75}Se-selenate, ^{75}Se-selenomethionine and ^{35}S-sulfate by rumen microorganisms in vitro. J. Anim. Sci. 27: 497-504.

Pearson, E. G. and Craig, A. M. 1980. The diagnosis of liver disease in equine and food animals. Mod. Vet. Pract. March pp. 233-237 and April pp. 315-320.

Pedersen, N. D., Whanger, P. D., Weswig, P. H. and Muth, O. H. 1972. Selenium binding proteins in tissues of normal and selenium-responsive myophathic lambs. Bioinorg. Chem. 2: 33-45.

Perry, T. W., Caldwell, D. M. and Peterson, R. C. 1976. Selenium content of feeds and effect of dietary selenium on hair and blood serum. J. Dairy Sci. 59: 760-763.

Peter, D. W., Whanger, P. D., Lindsay, J. P. and Buscall, D. J. 1982. Excretion of selenium, zinc and copper by sheep receiving continuous intraruminal infusions of selenite or selenomethionine. Proc. Nutr. Soc. Aust. 7: 178-181.

Peter, D. W., Young, P., Buscall, D. J. and Whanger, P. D. 1985. Selenium retention and concentrations in sheep given selenite or selenomethionine - anomalies, an apparent explanation and implications. Pages 484-487 in C. F. Mills, I. Bremner and J. K. Chesters, eds. Trace elements in man and animals - TEMA 5. Commonwealth Agricultural Bureaux, Slough, United Kingdom

Peterson, P. J. and Spedding, D. J. 1963. The excretion by sheep of ^{75}Se incorporated into red clover (Trifolium pratense L.): the chemical nature of excreted selenium and its uptake by three plant species. N. Z. J. Agric. Res. 6: 13-23.

Pickup, J. F. and McPherson, K. 1976. Theoretical considerations in stable isotope dilution mass spectrometry for organic analysis. Anal. Chem. 48: 1885-1890.

Prohaska, J. R. and Ganther, H. E. 1977. Glutathione peroxidase activity of glutathione transferases purified from rat liver. Biochem. Biophys. Res. Commun. 76: 437-445.

Puls, R. 1981. Veterinary trace mineral deficiency and toxicity information. Publ. 5139. Agriculture Canada, Ottawa, Ontario.

Reamer, D. C. and Veillon, C. 1981. Determination of selenium in biological materials by stable isotope dilution gas chromatography-mass spectrometry. Anal. Chem. 53: 2166-2169.

Reamer, D. C. and Veillon, C. 1983. A double isotope dilution method for using stable selenium isotopes in metabolic tracer studies: analysis by gas chromatography/mass spectrometry (GC/MS). J. Nutr. 113: 786-792.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. and Hoekstra, W. G. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 588-590.

Sandholm, M. 1973. The initial fate of a trace amount of intravenously administered selenite. Acta. Pharmacol. et. Toxicol. 33: 1-5.

Scholz, R. W. and Hutchinson, L. J. 1979. Distribution of glutathione peroxidase activity and selenium in the blood of dairy cows. Am. J. Vet. Res. 40: 245-249.

Scholz, R. W., Todhunter, D. A. and Cook, L. S. 1981a. Selenium content and glutathione peroxidase activity in tissues of young cattle fed supplemented whole milk diets. Am. J. Vet. Res. 42: 1718-1723.

Scholz, R. W., Cook, L. S. and Todhunter, D. A. 1981b. Distribution of selenium-dependent and nonselenium-dependent glutathione peroxidase activity in tissues of young cattle. Am. J. Vet. Res. 42: 1724-1728.

Schwarz, K. and Foltz, C. M. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79: 3292-3293.

Smith, K. L., Harrison, J. H., Hancock, D. D., Todhunter, D. A. and Conrad, H. R. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J. Dairy Sci. 67: 1293-1300.

Soder, M. 1984. Average analyses of British Columbia Feeds 1969-1984. District: Fraser Valley. British Columbia Ministry of Agriculture and Food, Victoria, British Columbia.

Stewart, R. D. H., Griffiths, N. M., Thomson, C. D. and Robinson, M. F. 1978. Quantitative selenium metabolism in normal New Zealand women. Br. J. Nutr. 40: 45-54.

Sunde, R. A. and Hoekstra, W. G. 1980. Incorporation of selenium from selenite and selenocysteine into glutathione peroxidase in the isolated perfused rat liver. Biochem. Biophys. Res. Commun. 93: 1181-1188.

Swanson, C. A., Reamer, D. C., Veillon, C., King, J. C. and Levander, O. A. 1983. Quantitative and qualitative aspects of selenium utilization in pregnant and nonpregnant women: an application of stable isotope methodology. Am. J. Clin. Nutr. 38: 169-180.

Symonds, H. W., Sansom, B. F., Mather, D. L. and Vagg, M. J. 1981a. Selenium metabolism in the dairy cow: the influence of the liver and the effect of the form of Se salt. Br. J. Nutr. 45: 117-125.

Symonds, H. W., Mather, D. L. and Vagg, M. J. 1981b. The excretion of selenium in bile and urine of steers: the influence of form and amount of Se salt. Br. J. Nutr. 46: 487-493.

Tappel, A. L. 1987. Glutathione peroxidase and other selenoproteins. Pages 122-132 in G. F. Combs, Jr., O. A. Levander, J. E. Spallholz and J. E. Oldfield, eds. Selenium in biology and medicine. Part A. Van Nostrand Reinhold Co., New York.

Thomson, C. D. and Stewart, R. D. H. 1973. Metabolic studies of [^{75}Se]selenomethionine and [^{75}Se]selenite in the rat. Br. J. Nutr. 30: 139-147.

Thomson, C. D. and Stewart, R. D. H. 1974. The metabolism of [^{75}Se]selenite in young women. Br. J. Nutr. 32: 47-57.

Thompson, K. G., Fraser, A. J., Harrop, B. M. and Kirk, J. A. 1980. Glutathione peroxidase activity in bovine serum and erythrocytes in relation to selenium concentrations of blood, serum and liver. Res. Vet. Sci. 28: 321-324.

Thompson, K. G., Fraser, A. J., Harrop, B. M., Kirk, J. A., Bullians, J. and Cordes, D. O. 1981. Glutathione peroxidase activity and selenium concentration in bovine blood and liver as indicators of dietary selenium intake. N. Z. Vet. J. 29: 3-6.

Trinder, N., Woodhouse, C. D. and Renton, C. P. 1969. The effect of vitamin E and selenium on the incidence of retained placentae in dairy cows. Vet. Rec. 85: 550-553.

Ullrey, D. E., Brady, P. S., Whetter, P. A., Ku, P. K. and Magee, W. T. 1977. Selenium supplementation of diets for sheep and beef cattle. J. Anim. Sci. 46: 559-565.

Underwood, E. J. 1977. Pages 302-346 in Trace elements in human and animal nutrition. 4th ed. Academic Press, New York.

Underwood, E. J. 1981. Selenium. Pages 149-166 in Mineral nutrition of livestock. 2nd ed. Commonwealth Agricultural Bureaux, Age Bros. (Norwich) Ltd., London.

Van Rij, A. M., Thomson, C. D., McKenzie, J. M. and Robinson, M. F. 1979. Selenium deficiency in total parenteral nutrition. Am. J. Clin. Nutr. 32: 2076-2085.

Van Soest, J. P. 1963. A rapid method for determination of fiber and lignin. J. Assn. Offical. Anal. Chem. 46: 829.

Van Vleet, J. F. 1980. Current knowledge of selenium-vitamin E deficiency in domestic animals. J. Am. Vet. Med. Assn. 176: 321-325.

Van Vleet, J. F. and Boon, G. D. 1980. Evaluation of the ability of dietary supplements of silver, copper, cobalt, tellurium, cadmium, zinc, and vanadium to induce lesions of selenium-vitamin E deficiency in ducklings and swine. Pages 366-372 in J. E. Spallholz, J. L. Martin and H. E. Ganther, eds. Selenium in biology and medicine. AVI Publishing Co., Inc., Westport, Connecticut.

Vijan, P. N. and Leung, D. 1980. Reduction of chemical interference and speciation studies in the hydride generation-atomic absorption method for selenium. Analytica Chimica Acta. 120: 141-146.

Whanger, P. D., Pedersen, N. D., Hatfield, J. and Weswig, P. H. 1976. Absorption of selenite and selenomethionine from ligated digestive tract segments in rats. Proc. Soc. Exp. Biol. Med. 153: 295-297.

Whanger, P. D. and Weswig, P. H. 1978. Influence of 19 elements on development of liver necrosis in selenium and vitamin E deficient rats. Nutr. Rep. Inter. 18: 421-428.

Whanger, P. D., Weswig, P. H. and Oldfield, J. E. 1978. Selenium, sulfur and nitrogen levels in ovine rumen microoganisms. J. Anim. Sci. 46: 515-519.

White, C. L., Cloninger, R. W., Hansen, J. A., Hoekstra, W. G. and Pope, A. L. 1979. Species variations in copper-selenium interactions. Proc. Nutr. Soc. Aust. 4: 148.

White, C. L., Hoekstra, W. G. and Pope, A. L. 1981. The effect of copper and molybdenum on ^{75}Se-selenomethionine metabolism in sheep. Pages 561-563 in J. M. Gawthorne, J. McC. Howell and C. L. White, eds. Trace elements in man and animals - TEMA 4. Springer-Verlage, New York.

Wilson, L. G. and Bandurski, R S. 1958. Enzymatic reactions involving sulfate, sulfite, selenate and molybdate. J. Biol. Chem. 223: 975-981.

Wilson, P. S. and Judson, G. J. 1976. Glutathione peroxidase activity in bovine and ovine erythrocytes in relation to blood selenium concentration. Br. Vet. J. 132: 428-434.

Wolffram, S., Arduser, F. and Scharrer, E. 1985. In vivo intestinal absorption of selenate and selenite by rats. J. Nutr. 115: 454-459.

Wright, P. L. and Bell, M. C. 1966. Comparative metabolism of selenium and tellurium in sheep and swine. Am. J. Physiol. 211: 6-10.

Wu, S. H., Oldfield, J. E., Whanger, P. D. and Weswig, P. H. 1973. Effect of selenium, vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8: 625-629.

Yang, G. Q. 1985. Keshan disease: an endemic selenium-related deficiency disease. Pages 273-290 in R. K. Chandra, ed. Trace elements in nutrition of children. Nestle Nutrition, Vevey/Raven Press, New York.

8. APPENDIX

Table 1. Isotope composition of natural abundance Se † and enriched isotope preparations ‡ (atomic percentage)

Isotope	^{na}Se	Se-76 (194802)	Se-77 (194901)	Se-78 (199901)	Se-82 (195201)
^{74}Se	0.87	0.08	0.03	0.06	0.12
^{76}Se	9.02	96.48	1.20	0.77	0.24
^{77}Se	7.58	0.83	94.75	0.37	0.53
^{78}Se	23.52	1.06	2.37	97.27	0.74
^{80}Se	49.82	1.38	1.49	1.42	1.70
^{82}Se	9.19	0.17	0.16	0.11	96.66

† From the Handbook of Chemistry and Physics (1970).
‡ Specifications for enriched stable isotopes supplied by Oak Ridge National Laboratory, Oak Ridge, TN.